THE CHEMISTRY OF LEATHER MANUFACTURE

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THIS BOOK

IS

DEDICATED TO THE MEMORY

OF

JOHN ARTHUR WILSON

(1890–1942)

GENERAL INTRODUCTION

American Chemical Society Series of Chemical Monographs

By arrangement with the Interallied Conference of Pure and Applied Chemistry, which met in London and Brussels in July, 1919, the American Chemical Society was to undertake the production and publication of Scientific and Technologic monographs on chemical subjects. At the same time it was agreed that the National Research Council, in cooperation with the American Chemical Society and the American Physical Society, should undertake the production and publication of Critical Tables of Chemical and Physical Constants. The American Chemical Society and the National Research Council mutually agreed to care for these two fields of chemical development. The American Chemical Society named as Trustees, to make the necessary arrangements for the publication of the monographs, Charles L. Parsons, secretary of the Society, Washington, D. C.; the late John E. Teeple, then treasurer of the Society, New York; and Professor Gellert Alleman of Swarthmore College. The Trustees arranged for the publication of the A.C.S. series of (a) Scientific and (b) Technologic Monographs by the Chemical Catalog Company, Inc. (Reinhold Publishing Corporation, successors) of New York.

The Council of the American Chemical Society, acting through its Committee on National Policy, appointed editors (the present list of whom appears at the close of this introduction) to select authors of competent authority in their respective fields and to consider critically the manuscripts submitted.

The first monograph of the series appeared in 1921. After twenty-three years of experience certain modifications of general policy are indicated. In the beginning there still remained from the preceding five decades a distinct though arbitrary differentiation between so-called "pure science" publications and technologic or applied science literature. This differentiation is fast becoming nebulous. Research in private enterprise has grown apace and not a little of it is pursued on the frontiers of knowledge. Furthermore, most workers in the sciences are coming to see the artificiality of the separation. The methods of both groups of workers are the same. They employ the same instrumentalities, and now frankly recognize that their objectives are common, namely the

search for new knowledge for the service of man. The officers o Society therefore have combined the two editorial Boards in a s Board of twelve representative members.

Also in the beginning of the series, it seemed expedient to con rather broadly the definition of a monograph. Needs of workers h be recognized. Consequently among the first one hundred monogr appeared works in the form of treatises covering in some instances r broad areas. Because such necessary works do not now want for lishers, it is considered advisable to hew more strictly to the line o monograph character which means more complete and critical treat of relatively restricted areas, and where a broader field needs cove to subdivide it into logical sub-areas The prodigious expansion of knowledge makes such a change desirable.

These monographs are intended to serve two principal purposes: to make available to chemists a thorough treatment of a selected ar form usable by persons working in more or less unrelated fields to th that they may correlate their own work with a larger area of phy science discipline; second, to stimulate further research in the sp field treated. To implement this purpose the authors of monograph expected to give extended references to the literature. Where the l ature is of such volume that a complete bibliography is impractic the authors are expected to append a list of references critically sel on the basis of their relative importance and significance

Preface

The purpose of this monograph is two-fold. It is designed primarily to summarize and to appraise our present scientific knowledge of the conversion of animal skin into leather. Its other purpose is to serve as a memorial to the life and work of a gifted pioneer leather scientist, John Arthur Wilson, the author of its first two editions, published in 1923 and 1929 respectively.

The chemistry of leather manufacture is actually the chemistry of connective tissue, of the organic tanning materials, of various inorganic tanning agents, and finally of the reactions that occur between skin proteins and tanning agents whereby leather is formed. This being true, progress in the so-called pure chemistry of these various fields has contributed directly to the progress of tanning technology. In turn, the advances within recent years in the chemistry of tanning have happily and significantly contributed to the sum of general scientific knowledge. It is for this reason that we confidently hope this book may prove of interest outside of its more restricted field.

The present need for this monograph has been accentuated by the war effort. Leather is an indispensable article in times of peace, and becomes a critical material during war. Coincident with the expanded war need, there has occurred a sharp diminution of the normally large importations of hides and skins and tanning materials. This difficult situation can be met only by a better utilization of domestic materials. The technical men of the industry whose problem this is—realize that better utilization is possible only through scientific knowledge. And they further realize that when peace is restored the leather industry will face grave competition from substitute materials.

A scientific field so complex and so far-flung as that of tanning lends itself to many, and often seemingly conflicting, viewpoints. Recognizing this, we have been primarily concerned with the presentation of experimental evidence and pertinent data, believing that while interpretations may change, the data remain vital. At the same time, we have sought to present adequately current and significant theoretical interpretations.

In order to expand certain subjects and yet remain within the limits of one volume, we have had to omit some of the subjects considered in the two volumes of the second edition. These omissions relate more particularly to descriptions of dyes and finishes, patent leather, furs and microscopic and histologic methods. We have also greatly reduced the number of illustra-

tions dealing with the histology of skin. All these omitted subjects are of interest and value and they may be found, as noted, in the second edition.

Each chapter of this volume was submitted to various colleagues especially qualified to criticize it. In this way we received many valuable suggestions from the following friends; R. S. Adams, D. H. Cameron, F. L. DeBeukelaer, J. H. Highberger, F. O'Flaherty, A. Schubert, G. W. Schultz, H. G. Turley and H. R. Wilson. Our special thanks are due Doctor Max Bergmann who read and criticized the entire manuscript. Nor would the senior author fail to express his appreciation of the time and facilities provided him by the B. D. Eisendrath Tanning Company of Racine, Wisconsin.

The chapter on the chemical composition of skin was written by John H. Highberger; that dealing with syntans by Karl F. Ruppenthal; the chapter on fatliquoring by Ralph E. Porter; and that on the physical testing of leather by Warren E. Emley.

For the loan of cuts and plates we are indebted to the *Journal of the American Leather Chemists' Association.*

G. D. McL.
E. R. T.

New York, N. Y.
April, 1945

Contents

Chapter 1

Introduction

If we list those materials necessary for human activity, leather is found to occupy an important place. It is therefore not surprising to find that tanning was probably the first manufacturing process in which man engaged, since both the clothing and protective armor of primitive man were of leather. Thus leather has been an indispensable item throughout history and is necessary to modern civilization in the form of shoes, clothing, harness, belting, and endless other useful articles.

All leather is made by treating animal skin with a tanning agent whereby the resulting product possesses qualities that are quite different from those of the original skin. The moist skin which has been removed from the animal's carcass is quickly decomposed by bacterial enzymes; it is rapidly dissolved by warm water; and if it is dried out, it becomes hard and largely useless. But when properly tanned, the skin is practically impervious to digestion by enzymes; it is insoluble in water and may be given whatever degree of softness is desired. It is the purpose of the following chapters to describe and to explain what is known of the principles which underlie that interesting and exceedingly complex reaction known as tanning.

A marked change has occurred in the leather industry during the past generation. Thirty years ago there were approximately 740 tanneries in the United States compared with 440 today, although the present volume of leather produced is considerably greater. This centralization of production went hand in hand with the growth and application of tanning science, and the unscientific tanner was unable to survive. The present tanning generation faces an even greater test, because many leather-substitute materials have appeared and others are being studied by competent scientific minds. It is a hopeful sign, however, that the leather industry is becoming increasingly aware that empirical methods must be replaced by scientifically sound procedures. This is evidenced by the fact that many research institutions devoted to the study of tanning have been founded throughout the world. But many tanning concerns are still unaware of their need for scientifically trained staffs; without such personnel they cannot hope to maintain their position in the competitive struggle which lies ahead. The most "practical" and successful tanner is he who not only respects the scientific approach but who is impatient with any other.

While each phase of the tanning process will be discussed in its relation to the end product, leather, it will be helpful at this point to summarize briefly the general steps involved.

Skins are, of course, produced all over the world and under a great variety of conditions. In any case the skin must be cured; that is, it must be so treated as to reduce or prevent its digestion by the many proteolytic bacteria which are present when the skin is flayed. This curing may be accomplished in certain cases by merely drying the skin, in other cases by partially dehydrating it with common salt, and in still other cases, by a combination of drying and salting. Light-weight skins, like those from small animals such as goats or sheep, or from immature animals such as calves, are referred to in the leather industry as "skins." Heavy-weight skins coming from large, mature animals such as steers, cows, or buffalo are termed "hides." When the term *skin* is used in this summary, it is employed in its inclusive sense and refers to both light and heavy skins.

When the cured skin reaches the tannery, it is "soaked." That is, it is treated with water to rehydrate it and to remove surface dirt, undesirable proteins, and the curing salt, in the case of salted skins.

After soaking, the skin receives the "unhairing" treatment. That is, it is placed in a solution of a chemical that will decompose the epidermal tissues and thus permit mechanical removal of the hair, while it partially saponifies the skin fat and produces desirable chemical and physical changes in the actual skin substance as well. The unhairing agent employed is usually a saturated solution of calcium hydroxide, to which various other chemicals known as accelerators are generally added. The time required for the unhairing process varies with the kind of skin and its condition, together with the nature of the unhairing solution and process and may range from one to ten days. Unhairing may also be accomplished by treating the soaked skin with certain enzymes or by "sweating," in which case the soaked skin is hung in a warm, humid room until the proteolytic bacteria it contains have digested its epidermis. But the great bulk of all skins are unhaired by the "liming" process, noted above. The only leathers not passing through the unhairing process are those included under furs; these are not considered in this book.

After the unhairing process, the skin is mechanically treated to remove the loosened hair, the disintegrated epidermis, much of the saponified animal fat, and also, the flesh, muscle, and adipose tissues adhering to the skin surface that was next to the animal's body. This side of the skin is termed its "flesh" side, while the outside surface which contained the hair is referred to as the "grain" side.

The skin is now delimed by soaking it in a solution of a chemical that will combine with the lime present in the skin to form a soluble lime salt,

which is then washed out with water. After deliming, the skin is "bated"—that is, it is usually treated with an aqueous infusion of an enzyme. This treatment brings about certain necessary chemical and physical changes in the skin. The skin is then ready for the tanning process.

In the case of heavy hides that are to be vegetable tanned, the bating treatment referred to above may be greatly curtailed in comparison to that given light skins, or it may be omitted entirely. In either event the heavy hide is placed in a very weak tannin infusion which is mellow in nature and in action; that is, it lacks astringency. This tan liquor is gradually increased in strength and astringency, until after a period of days, weeks, or months the hide is said to be tanned. Light skins that are to be tanned with either organic agents (such as the vegetable tannins) or with inorganic agents (such as the salts of chromium) are bated much more extensively than in the case of heavy skins or hides. This is because of the very different leather qualities desired in light leathers, such as shoe uppers, compared with heavy leather, such as sole.

When light skins are to be chrome tanned, they are first "pickled"; this means they are treated with a solution containing sulfuric or hydrochloric acid and sodium chloride. The acid rapidly combines with the skin protein and would cause it to swell were it not for the restraining action of the salt. The object of pickling is to bring the entire skin to a uniform chemical and physical condition and, also, to prevent the too rapid combination between skin substance and astringent chromium compound. The pickled skin is now agitated with a solution of a basic chromium compound, usually chromium sulfate, until it is considered to be tanned; this condition is coincident with a marked heat stability of the chrome tanned leather, which may be unaffected even by boiling water. The chrome tanning process is very much more rapid than vegetable tanning.

The chrome tanned skin is next treated with a mild alkaline solution to remove any unbound or free acid it may contain; this process is termed "neutralizing." After neutralizing the skin is dyed.

At the completion of heavy-leather vegetable tannage, and after neutralization and dyeing in the case of chrome tannage, the leathers are treated with the proper amount and kind of fatty materials; this process is termed "oiling" or "stuffing" in the case of heavy leathers and "fatliquoring" in the case of light leathers. The purpose of the process is to restore to the hide or skin the equivalent of the natural fatty materials that were saponified and removed by the liming process, since the finished leather would otherwise lack tensile strength and the proper elasticity.

After oiling or fatliquoring, the leather is dried and is ready for numerous "finishing" operations. These include various mechanical treatments, as well as the application of additional coats of coloring materials in the case of dyed leathers.

All the foregoing processes are further described and discussed in books dealing with the practical procedures of tanning, to which we refer the interested reader. The purpose of this volume is to discuss the more theoretical phases of tanning, since the future progress of leather manufacture is directly related to the extent to which scientific procedure replaces empirical practice.

Chapter 2

Histology of Skin

Our understanding of all of the processes involved in tanning has been enhanced by histological studies; and it may be said, in fact, that without a minimum of such knowledge we cannot hope to study or control tanning processes intelligently. But it must be pointed out that leather histology is a distinct science, requiring special technique and long experience in preparing and in interpreting skin sections, and that the lack of such knowledge has often led to disappointing and unfortunate results. Elaborate histological studies of human skin have been made, and these have greatly aided the leather histologist. But modern experience has shown that while there are many similarities between human skin and that of the animal skins used in tanning, there are also many differences. It is these differences, as well as the changes induced by the processes of tanning, which make necessary a distinct histology of tanning.

The whole subject of leather histology has been dealt with at great length by Wilson in the second edition of this monograph, to which the interested reader is referred, since lack of space prevents our giving more than a brief outline of the subject.

The first adequate histological studies of animal skin and leather were probably those of Boulanger[1] in 1908, but these were unfortunately published in journals not generally accessible to leather scientists. In 1917-21 Alfred Seymour-Jones[5] published a series of important articles which were valuable in themselves and which furnished stimulus to other investigators as well. Among the more recent general histological studies, and in addition to those of Wilson[5] already mentioned, are those of Turley,[7] Küntzel,[2] O'Flaherty and McLaughlin,[4] Theis and Serfass,[6] and D. J. Lloyd[3] and her collaborators.

Animal skin is made up of a number of distinct tissues and contains a number of distinct organs, as would be expected when the several physiologic functions of skin are considered. The tissues may be divided into the following classes: epithelial, connective, muscular, nervous, glandular, fatty, and the blood tissues. The organs include: voluntary and involuntary muscles, fat glands, sweat glands, nerves and blood vessels. These organs are illustrated and designated in Plate 1.[4]

The physiologic functions of the skin are of very great importance. One of the main functions of the skin is to keep constant the temperature of the

body it covers; this it does by permitting loss of heat by means of the sweat glands, or retaining body heat, when necessary, by means of the fat glands which can automatically cover the surface of the skin with oil and thus reduce surface evaporation. The skin is one of the principal excretory organs of

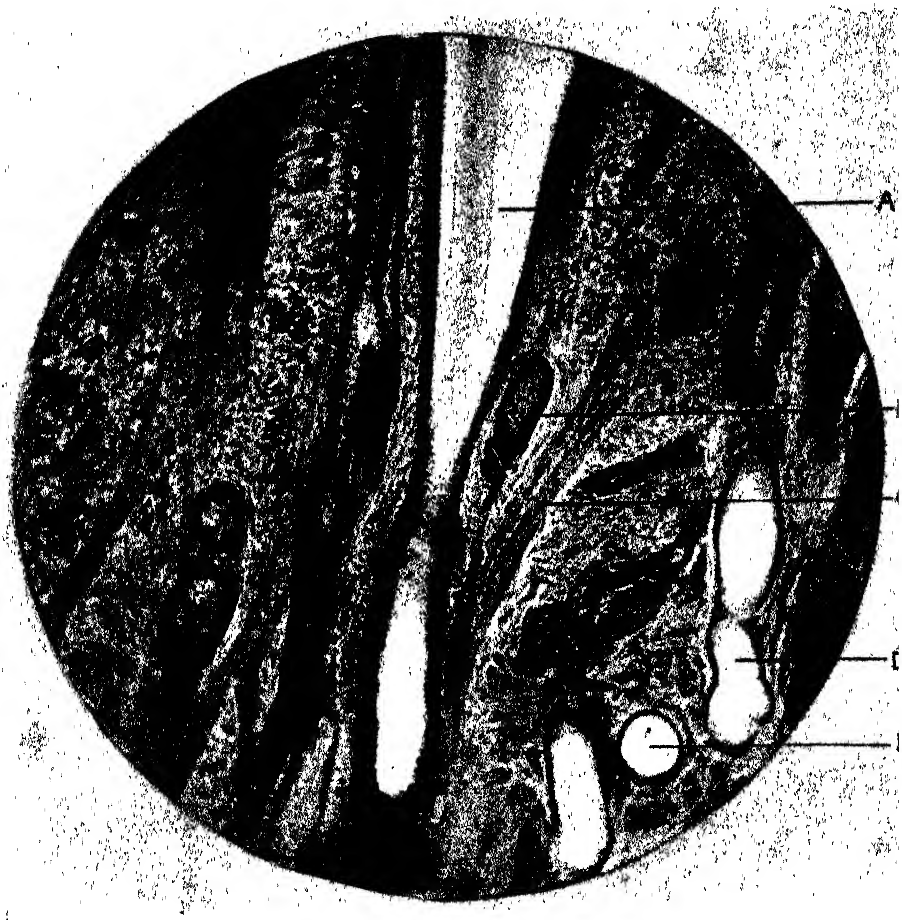

Plate 1. Frozen Section Steer Hide--Hematoxylin and Eosin Stain Showing:

A. Hair Follicle and Hair. C. Erector Muscle.
B. Oil Gland of Hair. D. Sweat Glands.

the body and protects it against bacterial invasion or mechanical dama as well. Another function of the skin is the development of what may termed color filters to protect underlying tissues from the harmful action the ultraviolet rays of the sun.

When the animal skin as received by the tanner is considered anatomically, it may first be divided into three distinct and superimposed layers: flesh, derma or corium, and epidermis. These three general layers are subject to further division, as described below.

Flesh. Strictly speaking, this tissue is not a part of the skin proper. Skin is attached to the underlying body by means of areolar connective tissue. When the skin is removed or flayed from the carcass, part of this areolar tissue remains attached to the skin, together with varying quantities of adipose tissue, yellow connective tissue, blood vessels, nerves, and voluntary muscle. All these tissues combined compose the "flesh," as it is expressed in tanning terminology. The flesh must be mechanically removed from the skin during its preparation for tanning, and this process is termed "fleshing." If the flesh were not removed, the diffusion of tanning materials or other chemicals into the skin from its flesh side would be impeded.

Derma. The derma, corium, or true skin, as it is variously designated, constitutes the leather-making material of skin, since both flesh and epidermis will have been removed prior to the tanning of the skin. The derma may be divided into an upper portion and a lower portion. The upper contains glands, muscles, and hair follicles, and has been aptly designated the "thermostat layer" by Wilson,[8] since it contains those organs concerned with the regulation of body temperature. The lower portion of the derma is usually termed the "reticular" layer, since the interlacing collagen fibers of which it is composed present a net-like appearance. The proportion which the thermostat layer bears to the total derma thickness varies greatly with the age of the animal. We have found that, as a general rule, the thermostat layer of a young calf, for example, represents a very much greater proportion of its derma thickness than in the case of the skin of a matured steer or cow. And we have noted that these proportionate differences seem to be a function of increased thickness of the reticular layer in the older animals, the actual thickness of the thermostat layer tending to remain approximately constant regardless of the animal's age.

The chemical constituents of skin are discussed in Chapter 3. But it will be well at this point to note that the main protein constituent of both dermal layers is collagen, which is arranged in interlacing bundles of fibers or fibrils. Seymour-Jones[5] suggested that the fiber bundles were enclosed in very thin sheaths of a substance he termed "fiber sarcolemma." These sheaths were later demonstrated by Turley.[7] Kaye and Lloyd[8] have shown photomicrographically that when collagen fibers of fresh skin are swollen in acidic or alkaline solutions, they show constrictions which seem to be due to tiny encircling threads of reticulin. The derma also contains a smaller amount of yellow connective tissue fibers composed of elastin. These fibers are located mainly at the lower and at the upper surfaces of the skin. Their

function is not clearly understood; it may be that those located near the upper or grain surface, and in close proximity to the erector pili muscles, serve to restore surrounding tissues to their normal position after they have been brought to a "goose-flesh" condition by the contraction of the erector pili muscle.

The connective tissue fibers of the thermostat layer are finer than those of the reticular layer, and they become increasingly finer as the epidermis

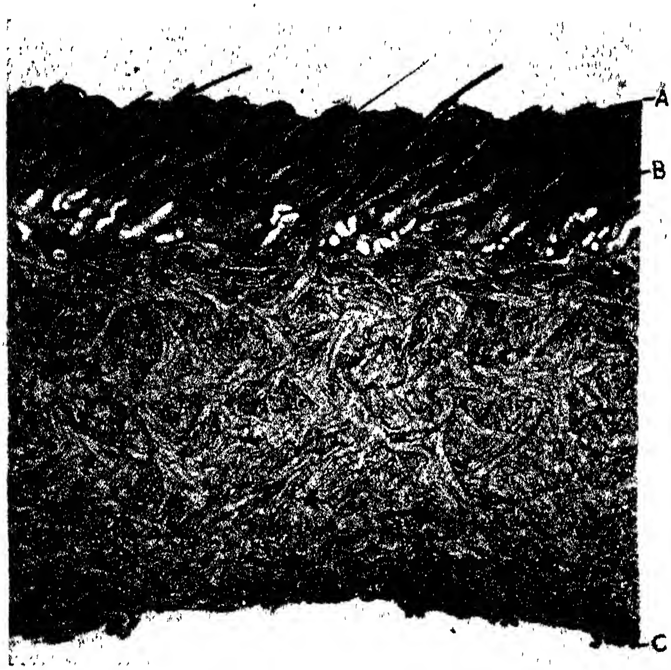

Plate 2. Frozen Section of Normal Steer Hide Stained with Scarlet R Fat Dye.

A. Epidermis—Outer layer Stratum Corneum Red
B. Sebaceous Glands and Ducts into Hair Follicles.
C. Fat of Subcutaneous Tissue.

(This hide was well fleshed during flaying, leaving very little flesh)

is approached and eventually become exceedingly fine and tend to lie parallel with the grain surface.

That portion of the derma immediately in contact with the epidermis has been designated the "grain membrane" by Seymour-Jones.[5] This grain membrane constitutes the grain surface of the finished leather. It has long been thought that the grain membrane is separated from the *stratum germinatum* (the lowest layer of the epidermis) by an extremely thin film, called the "hyaline layer." Küntzel[2] does not believe in the existence of this

layer, which he assumes to be nothing more than a particularly fine fiber interweaving at the uppermost derma surface. Turley[7] has, on the other hand, been able to quite definitely demonstrate a distinct band of about five microns thickness covering the fibers of the derma surface and lining the hair follicles as well.

Epidermis. Like the derma, the epidermis is made up of superimposed layers. Whereas the principal constituent of the derma is collagen, that of the epidermis is keratin. The two proteins are of very different composition and, fortunately for the tanner, respond very differently to treatment with unhairing solutions. When skin is limed, the epidermis is decomposed, thus enabling the mechanical removal of the hair, whereas the underlying collagen is not adversely affected.

As will be noted in Plate 2,[4] the epidermis comprises only a very small proportion of the total of the skin thickness and may, in one sense, and despite its importance, be regarded as a parasite. That is, it possesses no blood vessels of its own and draws upon the blood and lymph of the underlying derma for its nourishment. It grows by means of reproduction of its own cells.

That portion of the epidermis immediately in contact with the derma (or the hyaline layer) is termed *stratum germinatum*; it is also called the Malpighian layer. It is composed of elongated epithelial cells; these cells increase in height and then subdivide, one above the other. As subdivision continues, the older cells are pushed upward, away from nourishment, and consequently lose their power of reproduction until, finally, they become dehydrated and lifeless and are gradually worn away at the outer surface of the epidermis. The successive layers of epidermal cells have been termed: *stratum germinatum*, *stratum granulosum*, *stratum lucidum*, and the outermost, *stratum corneum*. They are illustrated in Plate 3.[4]

We may thus summarize the various layers of skin and their relative positions:

Epidermis	*Derma*
stratum corneum	hyaline layer
stratum lucidum	grain membrane
stratum granulosum	thermostat layer
stratum germinatum	reticular layer
	flesh

Küntzel[2] has questioned the correctness of any rigid classification of epidermal layers, pointing out that we are dealing with a continuous cell migration, which finally ends with the dead cell falling from the epidermal outer surface. He contends that this migratory process does not occur in layers, since an individual cell may differ greatly from its neighboring cells in its migratory rate. Küntzel believes that a layer-like cell arrangement in the epidermis is to be found only in the stratum germinatum.

Plate 3. Epidermis of Steer Hide.

A. Stratum Germinatum.
B. Kerato-Hyaline Granules in Cells of Stratum Granulosum.
C. Optically Appearing Empty Space—the Stratum Lucidum.
D. Flat Dead Cells of the Outer Coat—the Stratum Corneum.

Animal hair, like nails, hooves, scales, and feathers, is an epidermal growth. Noting the hairs shown in Plate 2, it is seen that the part of the hair which is below the skin surface rests in a pocket, or follicle. This follicle is shown in greater detail in Plate 3. That part of the hair projecting beyond the surface of the skin is called the hair shaft, and that below the surface the hair root. The hair root penetrates deeply into the derma, the depth of penetration varying with the species of animal. The hair follicle is composed of epidermal tissues on its inside and of dermal tissue on its outside. The lower and thickened part of the hair root (see Plate 1) is called the hair bulb. The base of the follicle is penetrated from below by a projection arising from the derma and known as the hair papilla. The papilla is plentifully supplied with nerves and with blood vessels which supply its nourishment. Epithelial cells line the lymph space surrounding the papilla. As long as these cells form, the older cells are pushed outward through the follicle and form the hair. When the cells cease to form, or if the papilla blood vessels fail to furnish cell nourishment, baldness results.

As stated above, we have not attempted in this chapter to give more than a very general outline of the intensely interesting subject, skin histology. The subject is dealt with at length in the second edition of this monograph and by the numerous workers already referred to.

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Chapter 3

The Composition and Chemical Structure of Skin

Animal skin, like other tissues of the body, is composed of proteins, lipids, carbohydrates, mineral salts, and water. During the past fifteen years important advances have been made in our knowledge of the chemical composition of skin, particularly with reference to the proteins and lipids. In this chapter an effort will be made to summarize the available knowledge, and to present a general picture of the skin's composition and chemical characteristics. The principal constituents will be discussed separately under the classifications noted above. Brief discussions of the skin pigments and more important skin enzymes are also included.

Proteins of the Skin

The solid matter of the skin is made up preponderantly--from about 90 to about 95 per cent--of proteins. In the whole fresh skin they comprise roughly 35 per cent of the weight. Several classes of proteins are represented, the most important of which are collagen, elastin, keratins, glycoproteins, albumins, and globulins. Of these, those of greatest importance to the tanner—essentially the first three named--are *fibrous* in nature, while the others belong to the class of *globular*, or *corpuscular*, proteins. The division of all proteins into these two broad categories has come into use in recent years. It emphasizes the reflection in their properties and structures of what is essentially a functional difference between the two types. The globular proteins are in general those which are more intimately connected with the vital processes of the body. They function as the essential constituents of the nuclei and protoplasm of actively multiplying cells, as oxygen carriers in the blood, as food materials, and in other capacities where active metabolic processes are carried on. The primary function of the fibrous proteins, on the other hand, is in connection with various structural features of the body. They are found where strength, together with flexibility or elasticity, is required in supporting or structural elements, as in tendons, muscles, cartilage, etc., or where the function is primarily a protective one, as in the nails, hair, and skin. Although they are built up of essentially the same types of chemical units, the α-amino acids, nature has adapted the properties of these two groups of proteins to their respective functions in a remarkable manner. Thus the globular proteins are in general soluble, and

the fibrous proteins insoluble, in water or dilute aqueous systems. These differences in properties are undoubtedly achieved partially by variations in the proportions of the various amino acids or prosthetic groups composing them, but they seem primarily to be due to fundamental differences in the molecular organization of the structures.

Since the proteins of most importance in the skin from the tanner's standpoint are all fibrous, it will be necessary to consider in broad outline the present concept of fiber structure, developed as a result of the work of the last fifteen years, before discussing the individual proteins themselves.

The Modern Concept of Protein Fiber Structure

Following the pioneer work of Emil Fischer and his school in the closing years of the last and the early years of the present centuries, protein chemists were largely concerned with the development of the peptide theory of protein structure. According to this theory, which is now so firmly established as to be universally accepted, all proteins are essentially built up of α-amino acids joined together by the elimination of water between the carboxyl group of one and the amino group of another to form the *peptide linkage*. The resulting structures, as they exist in the proteins, are of very high molecular weight, and have the general formula:

$$(\quad NH\ CHR\ CO\ NH\ CHR\ CO\ NH\ CHR\ CO\ .\quad .)_x$$

On hydrolysis, which may be brought about by the action of acids, bases, or enzymes, these *polypeptide chains* are broken down into their constituent amino acids. For the first three decades of the present century protein chemists were primarily engaged in the determination and identification of the various amino acids isolated from such hydrolysates. A recent authoritative survey[137] lists twenty-five amino acids which are generally accepted as occurring in proteins. About twenty of these are quite widely distributed and are found in most proteins. In addition, a further group of twenty-two comprises those which have been found in nature, but have not definitely been proven to occur in proteins, as well as others which have been claimed by various investigators without substantiation. Many of the latter group are of doubtful authenticity. The accepted amino acids, exclusive of their common carboxyl and α-amino functional groups, are of the most diverse chemical nature. They range from the simple glycine to those carrying more complex hydrocarbon groups of an aliphatic (leucine, valine), or an aromatic (phenyl-alanine) nature, or phenolic groups (tyrosine), or heterocyclic rings (tryptophane), or excess basic (lysine, arginine, histidine), or carboxyl (aspartic and glutamic acids) groups. In the generalized polypeptide formula given above, where —NH·CHR·CO— is one amino acid *residue* as it is built into the chain, R represents the individual, distinctive

portion of the amino acid molecule. These R groups, or *side chains*, as they are usually called, may thus be of widely different chemical character, from a single hydrogen atom to sizable chains which may bear strongly polar groups of acidic or basic function.

While the determination of the amino acid composition of the proteins continues to be an active and highly important field of work, beginning in about the middle 1920's investigators began to be interested in the possible configurations which might be assumed by the polypeptide chains, and the manner in which such chains might be arranged to form the total protein structure. A natural line of attack on this problem was the utilization of the powerful method of x-ray diffraction analysis, which had been shown by the Braggs and others to be capable of yielding complete structure determinations in the cases of simple crystalline materials. Since none of the globular proteins was at that time available in crystalline form, and x-ray photographs of ordinary protein preparations gave only hazy, "amorphous" rings, investigators turned to protein fibers, the definite macroscopic form and optical anisotropy of which gave promise of better results. X-ray diffraction photographs of such fibers, taken with the x-ray beam perpendicular to the long direction of the fiber, did indeed show reflections which, although not in general as sharp or as well defined as those given by simpler, highly crystalline materials, were nevertheless unquestionably the result of some sort of crystalline or semi-crystalline molecular arrangement. A further point of difference of these x-ray patterns from those of the simple crystals lay in their relative paucity in number of reflections. This fact, together with the lack of optical crystallographic data generally necessary for complete structure determinations, forced investigators to turn to other methods than the rigid mathematical processes of ordinary x-ray crystal structure analysis. In the present state of its development, the investigation of protein fiber structure by the x-ray method leads to satisfactory measurements of the interplanar spacings causing the reflections observed*; the identification of the spacings, and the interpretation of the structure in terms of them, however, rests in general upon inferences derived from observing the effects of alteration in physical and chemical states upon the diffraction patterns, and from analogies drawn from simpler, completely determined structures. The x-ray patterns do permit the direct deduction, however, that protein fibers are built up from long, thin crystallites arranged with their long axes more or less parallel to one another and to the long axis of the fiber, but of all possible orientations in other directions. These are the characteristic features of the structures of all fibers, regardless of their chemical nature. It is this type of structure that imparts to the fiber

* For a simple exposition of the principles of the x-ray method, see H. Kersten, *J. Am. Leather Chem. Assn.*, **31**, 84 (1936).

its strength and flexibility. Other physical properties are associated with the chemical nature and stereochemical configuration of the units making up the crystallites, as will be seen.

Investigators studying various protein fibers by the x-ray method have found three groups of spacings, or dimensions, which appear to be characteristic of the fibrous protein structure. The first of these is the spacing between planes which run transversely to the fiber axis; that is, it is the distance between identical* atomic groupings which are repeated in a direction parallel to the long axis of the fiber. In silk fibroin this distance is about 7.0 Å (1 Å = 1 Ångstrom Unit = 10^{-8} cm), and is interpreted as the length or two amino acid residues as they are built into the polypeptide chain. Such chains, owing to the possibility of free rotation about the single bonds of the carbon and nitrogen atoms, are able to assume various lengths, in all but the longest of which the chains are coiled or folded upon themselves

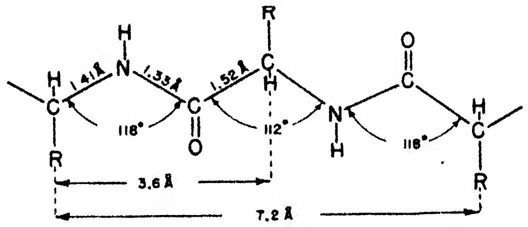
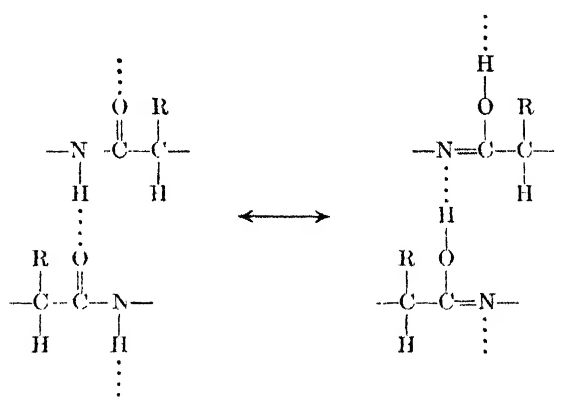


Figure 1. Interatomic distances and bond angles in a fully extended polypeptide chain.

to some degree. In the fully extended condition, owing to the fact that all of the amino acids belong to the same stereochemical configurational family (the *l*-configuration), the side chain R groups extend alternately from one side and the other of the main chain. The distance between two R groups on the same side of such a fully extended chain, calculated from interatomic distances and bond angles found in simpler, analogous compounds, checks well with the characteristic repetition distance observed along the fiber axis in the case of silk and other protein fibers where there is reason to believe that a fully extended condition of the chains exists. Figure 1 shows a diagrammatic representation of a segment of such a polypeptide chain. In such cases the length per amino acid residue is one-half the observed periodicity, or about 3.5 – 3.6 Å. In cases where the length per residue is found to be significantly smaller than this value, a coiled or folded condition of the chains is indicated. This condition is usually associated with some type of long-range elasticity in the fiber.

* This statement is not strictly correct, since it takes no account of the difference between the R groups. The true *unit cell* (of which the repetition distance along the fiber axis is one dimension) of such a structure is probably many times larger than the *pseudo* unit cell ordinarily found in these investigations.

The other two characteristic dimensions found in fibrous proteins are both associated with the fiber cross-section; the spacings are repeated in directions perpendicular to that discussed above, and to the fiber axis. They are therefore the distances between sets of planes containing like atomic groupings, and running lengthwise of the fiber. Of these two dimensions, the first, amounting to about 4.5 Å, is approximately the same for all proteins. It is interpreted as the lateral distance between neighboring polypeptide chains, and is determined by the closeness of approach of the main chains to each other. Since the groups making up the backbones of the chains are common to all proteins, the fact that this spacing is nearly constant is readily understood; it is usually termed the *backbone spacing*. The chains may thus be conceived of as arranged in layers, in each of which the successive chains are separated from each other by about 4.5 Å. The cohesion between the chains is probably due to hydrogen bridges formed between CO and NH groups in neighboring chains. Such NHO bridges would be expected to occur by analogy with simpler structures, and their definite existence in proteins is attested by the evidence of infrared spectroscopy.[27] The stability of such linkages in the proteins is probably greatly enhanced by resonance between the forms



and by synchronized oscillation of the bridging hydrogens along the long chains.[56,58]

The second lateral dimension varies somewhat from protein to protein and, what is more striking, in certain cases, notably in collagen and gelatin, it varies in the same protein, depending upon the moisture content. This spacing, which is about 10 Å in many dry proteins, is interpreted as the distance to which the layers containing the main chains are separated by the projection of the side chains. Accordingly it is usually termed the *side chain spacing*. This distance will obviously be largely dependent upon the chemical nature of the amino acids composing the individual protein.

Figure 2 shows a diagrammatic representation of the production of x-ray reflections by the 7.0 Å spacing, and the backbone spacing, in the case of an idealized structure formed of layers of fully extended polypeptide chains. In order to avoid complicating the figure unduly the production of the side chain lateral reflection is not shown. The structure represented in the

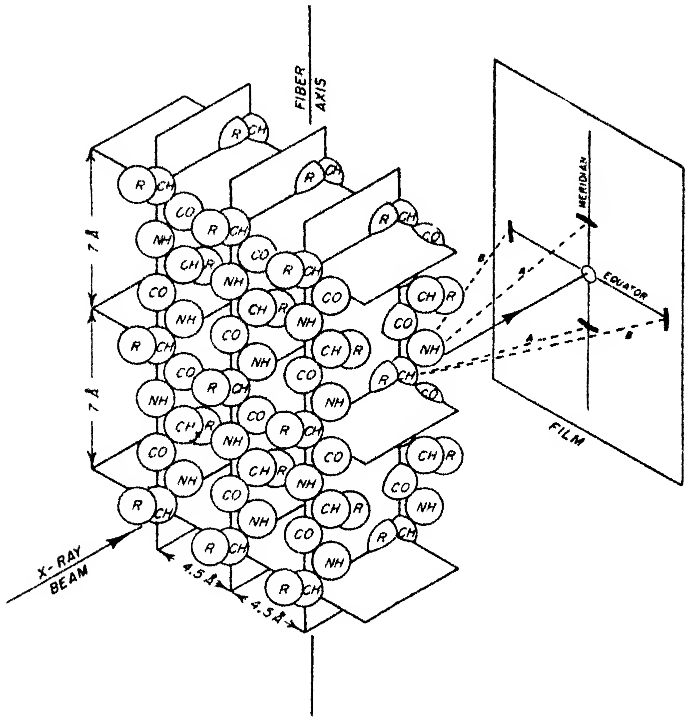


Figure 2. Illustrating the formation of a fiber diagram by the diffraction of parallel, monochromatic x-rays by an idealized protein structure composed of fully extended polypeptide chains, when the x-ray beam is perpendicular to the fiber axis. The diffraction may be considered as a *reflection* from hypothetical planes passing through like atom groups. The angle of incidence of the x-ray beam with the planes, and the distance apart of the latter, must satisfy Bragg's law relating these quantities to the x-ray wavelength, in order for "reflection" to occur. A = beams reflected from transverse planes, producing diffraction spots on the meridian of the film; B = beams reflected from parallel planes, producing spots on the equator.

segment of fiber crystallite shown is essentially (with minor variations) that proposed for silk fibroin by Meyer and Mark,[91] in one of the pioneer comprehensive investigations in this field. It must be emphasized, however, that the x-ray diagram actually produced by silk is quite different from the over-simplified sketch shown, which is intended only to illustrate the principles of the formation of a fiber diagram. The fact that arcs, rather than

sharp spots, are found on most x-ray fiber photographs, is due to the divergence from exact parallelism of the crystallites. As is shown in Figure 2, planes transverse to the fiber axis produce reflection spots or arcs on the meridian of the film, under the conditions shown, while those produced by planes parallel to the fiber axis appear on the equator. It is usually observed that the reflections on the meridian are much sharper and more clear-cut than those on the equator. This is a consequence of the fact that there is a far greater number of diffracting planes transverse to the fiber axis than parallel to it, and leads to the conclusion that the crystallites of the fiber are much longer in the direction of the fiber axis than they are thick in the cross-section of the fiber.

A further important phenomenon observed in the x-ray investigation of protein fibers must be noted. The preceding discussion has dealt with patterns produced when the x-ray beam is directed normal to the fiber axis; such patterns consist of spots or arcs. When the beam, however, is directed parallel to the fiber axis an altogether different result is obtained. Under these conditions the resulting diagram consists of whole concentric circles or rings of appreciable width, usually not more than one or two in number. This production of complete circles is a consequence of lack of preferred orientation of the crystallites in other directions than parallel to the fiber axis. The crystallites, while all arranged with their long axes parallel to each other and to the fiber axis, are thus of all possible orientations in other directions.

In the x-ray diffraction patterns of all natural protein fibers, the crystalline reflections are obscured to a varying degree by a "back-ground haze" arising from amorphous matter. The nature of these amorphous regions, and their relation to the crystalline portion of the structure is not yet clear. Whether the crystallites are to be considered as imbedded in an amorphous phase of the same, or of different, chemical composition, or whether the long polypeptide chains are brought together in an ordered arrangement in certain regions, while being merely entangled into an amorphous mass in others ("fringe theory" of Hermann, Gerngross, and Abitz[51]) is still not clear.

The essential features characteristic of the general structure of protein fibers are, however, fairly apparent. Astbury speaks of such fiber structures as a kind of "molecular yarn," and this phrase seems to characterize the general structure and properties extremely well. With this general picture in mind, we may now proceed to discuss the individual proteins of the skin.

The Keratins

The keratins are the fibrous proteins which compose the outer layers of the skin and its appendages. The term covers a group ranging from hair, nails, and other horny structures to the proteins of the softer tissues of the

lower epithelial layers, where the essentially fibrous character is not so readily recognizable. In addition to the obvious differences in physical properties, some difference in chemical properties exists between these various keratins; in the tannery process of unhairing, for instance, the keratinous matter composing the epithelial layers is attacked by the alkaline solution before the hair itself is visibly affected. These softer keratins are also more susceptible to attack by enzymes. The probable cause of such differences will be discussed later.

The keratins which have usually been chosen for chemical study are those of hair, wool, nails, or horn. They are insoluble in water or dilute acids or alkalies, although attacked slowly by moderately strong solutions of the latter in the cold. They are quite resistant to the action of the ordinary enzymes such as pepsin and trypsin, although, as noted above, this statement does not apply to the softer keratins. Sookne and Harris[121] have determined the isoelectric point of wool keratin electrophoretically as pH 4.2 in acetate buffers. Keratin in the form of hair or wool fibers swells very little over practically the whole pH range. After treatment with alkaline solutions, however, the swelling is increased, and two maxima appear, at pH values of about 2 and 10.[60,123] It is probable that the softer keratins have a much greater swelling capacity.

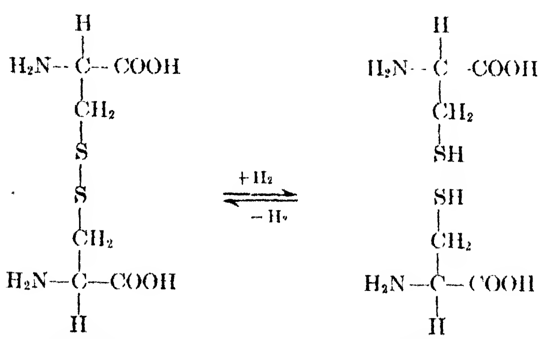
Keratin is usually prepared by extracting horny tissues with alcohol and ether to remove fats, then subjecting the material to digestion with an acid pepsin solution, followed by an alkaline trypsin solution. Both digestions are allowed to proceed for about a week. The material is then re-extracted with alcohol and ether, and dried at room temperature.

The amount of keratin in the skin naturally varies considerably, both with species and probably also with age. The thickness of lighter skins is usually decreased more at the expense of the derma than of the epidermal system, so that in such skins the proportion of keratin is correspondingly higher. Rosenthal[111] has reported from 5.28 to 6.41 per cent of the dry matter of dog skin to be keratin, depending upon location of the samples. Similar results for calf skin are given as from 19.91 to 36.15 per cent. The latter figures seem extraordinarily high, but these data must in any event be treated with considerable reserve, since Rosenthal's methods seem to have been scarcely adequate to effect clean-cut separations of the various protein materials.

The amino acid composition of keratins from various sources, as reported by different investigators, has varied considerably. While some of this variation is probably to be ascribed to experimental error and the different techniques used in the analyses, there is no doubt that the different keratins do differ to some extent in chemical composition. What are considered to be the most reliable figures for the keratin of wool fiber are given in Table 1.

The chief characterizing feature of the keratins has ordinarily been considered to be their relatively high content of the sulfur-containing amino acid cystine. The only other sulfur-containing amino acid known definitely to occur in the proteins, methionine (α-amino-γ-methylthiol-n-butyric acid), is present in only very small quantities in the keratins, and the total sulfur of wool keratin is almost completely accounted for by these two amino acids.[11,105] In recent years, however, Block has advocated the idea that the essential chemical characteristic of the keratins is the molecular ratios of the basic amino acids occurring in them, rather than the cystine content. In analyses of a considerable variety of keratins, Block and his co-workers have found approximately constant values for these ratios, although the actual weight percentages varied considerably, as is also true of the cystine contents. Accordingly, Block and Vickery[21] have proposed that a keratin be defined as "a protein which is resistant to digestion by pepsin and trypsin, which is insoluble in dilute acids and alkalies, in water and organic solvents, and which on acid hydrolysis, yields such quantities of histidine, lysine, and arginine, that the molecular ratios of these amino acids are respectively approximately as 1 : 4 : 12."

In spite of the fact that the cystine content of the keratins varies (although usually relatively high), this amino acid is undoubtedly of great importance in their structure, and is probably responsible, in part at least, for their characteristic properties. Cystine is unique among the known naturally occurring amino acids in being a twinned molecule of a simpler amino acid, cysteine, with which it forms a reversible oxidation-reduction system:



Cystine *2 mols of cysteine*

The question of which of these two forms actually occurs in the proteins is still a matter of discussion. Owing to the readily reversible transformation of one into the other, experimental difficulties are presented in an attempt to settle the question which have not yet been entirely surmounted. Until fairly recent years it was believed that cysteine did not occur naturally in the proteins, but recent evidence,[92] based on positive nitroprusside and other

tests for the free sulfhydryl group in certain proteins, indicates this to be incorrect. It is very probable that, at least in some cases, either or both forms may occur in the same protein, depending upon conditions.

The idea that cystine may participate in the formation of two different polypeptide chains, one-half of its molecule being built into each of two neighboring chains, with a resultant disulfide bridge between, and that this type of linkage may be of peculiar importance in explaining the resistant properties of the keratins, apparently was first suggested by Starey.[126] This conception has been used by Astbury[2] in connection with his suggested keratin structure to be discussed below, by Speakman,[122] and by others, and is now widely accepted, although it is to be remembered that it is not definitely proven by any evidence now at hand. The correlation of the insoluble, enzymatically resistant character of the keratins with their high cystine contents is, however, a logical one, and their chemical behavior also fits in well with the disulfide bridge hypothesis. The susceptibility of the keratins to attack by alkali sulfides, cyanides, and other reducing agents, as observed in the technical unhairing of skins, is to be explained as a reductive fission of these bridges. Goddard and Michaelis[42] have shown that wool keratin can be converted into a soluble form by reduction with thioglycolic acid, cyanides, or other reducing agents. Harris and his co-workers have recently studied this phenomenon extensively, and have advanced new chemical evidence in favor of the disulfide cross-linkage theory. They have shown[100] that wool in which the S-S linkages have been broken by reduction is almost completely digested by pepsin and chymo-trypsin (but is only slightly attacked by trypsin, however), and is also much more readily attacked by alkali. It is possible to carry out the reduction without visibly affecting the fiber structure. When the free SH groups are reconverted to disulfide groups by oxidation, the wool regains its original stability.

After the promising results obtained in the x-ray investigation of the simple silk structure, x-ray crystallographers turned to such fibers as hair and wool. With these the first results were discouraging, since the patterns obtained, although undeniably due to some type of partial, if imperfect crystalline organization, contained even fewer reflections than those of silk fibroin. Furthermore, there seemed to be no spacing or periodicity which bore any recognizable relation to those which might be expected from an assemblage of polypeptide chains. The principal features of the diagram obtained from wool[9] were the strong meridional arcs of spacing 5.15 Å, and the large equatorial spots of spacing 9.8 Å. A diagrammatic sketch of the pattern is given in Figure 3. Astbury and Street[9] state of this pattern that "it is what might be expected from an imperfectly crystalline system in which the only sharply defined translation is that parallel to the fiber axis; in other words, it suggests long filament-like molecules which cling together

sideways with varying degrees of perfection." It was evident that, aside from this adherence to the general principles of fiber structure, the keratin fiber was constructed on a different plan from that of silk.

The resolution of this difficulty came with the illuminating discovery by Astbury and Street[9] that hair or wool, when stretched to approximately double its original length by steaming the fiber while under tension, gave an entirely different x-ray photograph.* The new pattern began to appear when the fibers were about twenty to thirty per cent extended, and at about sixty to seventy per cent extension had almost completely replaced the original diagram. In the new pattern (see Figure 3) the meridional reflection of 5.15 Å was replaced by one of spacing 3.32 Å and periodicity 6.64 Å†, while on the equator strong new spots corresponding to a spacing of 4.65 Å appeared. The equatorial reflections of 9.8 Å remained in the new pattern, although somewhat decreased in size. The diagram of the stretched keratin, which Astbury has called β-keratin, the original form being termed α-keratin, is thus strikingly similar in its essentials to that of silk fibroin discussed in the preceding section. The periodicity in the direction of the fiber axis, 6.64 Å, and the corresponding amino acid residue length, 3.32 Å‡ (on the basis of two residues), are slightly less than in silk, but sufficiently close to be readily explainable by the difference in configuration of the main chains, produced by the bulkier side chains of keratin as compared to those of silk fibroin, which consists largely of glycine and alanine. If the Meyer and Mark structure for silk fibroin is accepted in its essentials, it is scarcely possible to avoid the conclusion, which Astbury and Street drew, that β-keratin is also formed of fully extended polypeptide chains held together by secondary valence backbone forces, and by primary

* About six years earlier J. R. Katz had observed the transformation of the x-ray diagram of rubber from an amorphous ring to a crystalline pattern on stretching. Katz, however, did not interpret his observations as indicating any fundamental change in the form of the molecules, but sought rather to associate them with a change from a random to a crystalline orientation, brought about by stretching. See his discussion of Astbury's paper in "The Colloid Aspects of Textile Materials and Related Topics," The Faraday Society, London, 1932, p. 207.

† Although it is not evident from the simplified sketches of Figure 3, nor from the ordinary photographic reproductions of the x-ray pictures, the strong meridional reflections in both pictures appear on the *second layer line*. The interpretation of fiber diagrams is based largely on analogy with rotating crystal photographs, in which layer lines are produced which give directly the *primitive translation*, or dimension of the unit cell, parallel to the axis of rotation. This periodicity, determined from a reflection on the nth layer line, and also on the meridian, is n times the observed spacing of the reflection. Owing to the fact that a fiber is an assemblage of crystallites of all possible orientations, except along the fiber axis, a photograph taken perpendicular to this axis produces essentially the same effect as is obtained on rotating a single crystal about one of its principal axes. Thus the "pseudo-primitive translations" in the present cases are 10.3 and 6.64 Å respectively, although the true values are probably some multiple of these. See footnote on page 15

‡ Astbury and Woods[10] state that the average value in β-keratin is 3.38 Å, and that the exact value is probably dependent upon the previous history and particular state of tension of the fiber.

valence side chain interactions, the respective distances between main chains being 4.65 Å and 9.8 Å.

Astbury and Street further concluded that the fully extended chains of β-keratin are formed from chains existing in a folded condition in the α-keratin, by some process of unfolding during the stretching of the fiber. This conception was strengthened by a comparison of the physical properties of silk and wool fibers. The extension of the wool fiber is elastic; on release of the tension under the proper conditions the fiber returns nearly to its original length, and the α-keratin x-ray diagram is simultaneously restored.* On the other hand, silk has a very low elastic extensibility beyond which it cannot be stretched without permanent deformation. Furthermore, silk fibers stretched even considerably beyond the elastic limit show no funda-

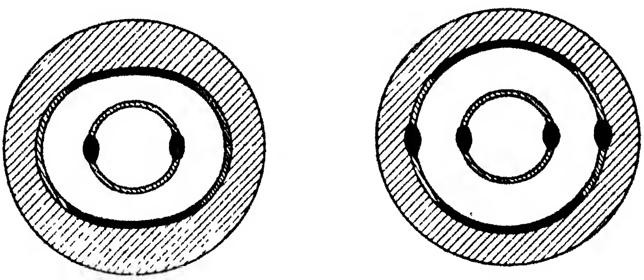


Figure 3. Diagrammatic sketches of x-ray diagrams from keratin. Fiber axis vertical. Left, α-keratin. Right, β-keratin. After Weidinger, *Coll.*, 837, 4 (1940).

mental change in x-ray diagram. It is a natural conclusion that the mechanism of the extension is entirely different in the two cases, and that in wool it is achieved by the elongation of what may be called a molecular spring, which contains powerful internal forces tending to restore it to its original shape, while in silk it is produced by the slipping over one another of chains which are already fully extended.

These ideas are accepted in principle, if not in detail, by most protein chemists.† The main debate has centered around the structure to be assigned to α-keratin, and particularly the nature of the folds in the chains, and the types of linkages operative in maintaining and restoring the folded condition. In Astbury's originally suggested structure, which he has described in numerous places,[4,5] the main chains were conceived of as folded into a series of hexagonal structures very similar to diketopiperazine rings. A layer of such chains held together by covalent side chain linkages (disulfide bridges and

* This is true only for fibers stretched in water. Steam produces an irreversible change in which the power of regenerating the original x-ray pattern is lost.

† See, however, the criticisms of Harrison,[49] who maintains that the different physical and chemical properties of the cuticle and the cortex of the fiber explain the phenomena observed.

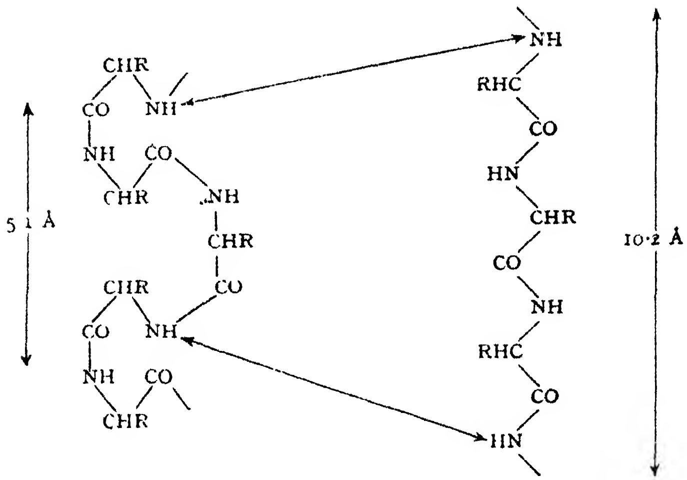


Figure 4. Hexagonal folding of the polypeptide chains of α-keratin, according to Astbury's original conception, and the production of the β- from the α-form by extension of the chains. From W. T. Astbury, *J. Textile Inst*, 27, 281 (1936).

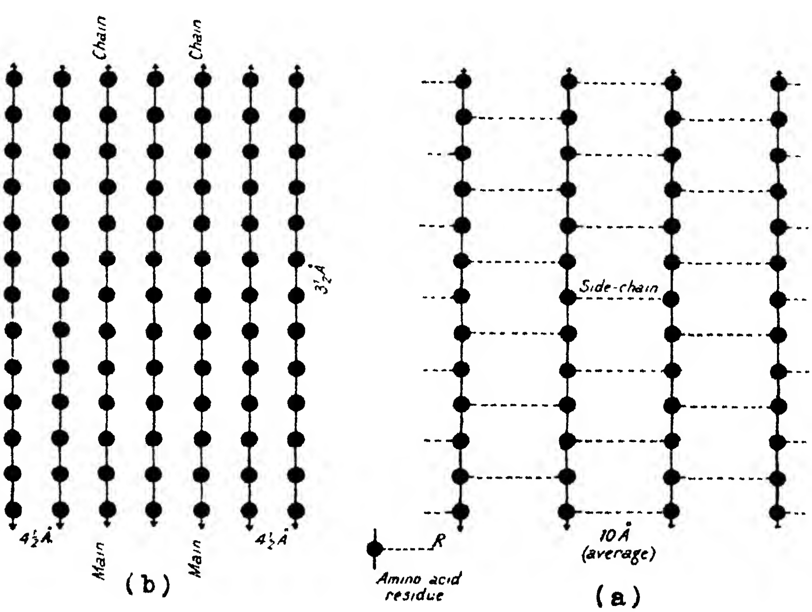


Figure 5. Diagrammatic representation of the structure of β-keratin, according to Astbury. (a) A grid of fully extended polypeptide chains. (b) Edge-on view of a stack of such grids. The α-form is derived from (a) by folding the paper transversely. From W. T. Astbury, *J. Textile Inst.*, 27, 281 (1936).

other covalent or electrovalent links, of average spacing 9.8 Å) constituted a "grid," and the total structure was formed of a stack of such grids cohering by the secondary valence forces along the backbones (4.5 Å). This structure and its relation to the β-keratin structure, is shown in the idealized diagrams of Figures 4 and 5. The periodicity observed along the fiber axis was associated by Astbury with three amino acid residues, which in the folded condition in α-keratin occupied about 5.1 Å, and became pulled out in the β-form to a length of about 10.2 Å, giving an average residue length in the latter case of around 3.4 Å. The elastic properties of the fiber were attributed to the fact that on extension the side chain linkages are subjected to a certain amount of strain, with a natural tendency to return to the original unstressed form on release of the tension. The phenomenon of "permanent set" in hair or wool was explained by Astbury as being due to the hydrolytic rupture of certain of these linkages, and their re-formation in an unstressed configuration, by the prolonged action of the hot water or steam. Astbury and Woods[10] also studied the "supercontraction" of hair or wool fibers, a contraction to about two-thirds of the original length, induced by releasing the tension immediately after stretching in steam. In such cases, according to Astbury and Woods, some of the side chain linkages are ruptured, but have no opportunity to re-form, and the chains are thus left in a more labile condition which leads to an even greater degree of folding than existed in the original form.

With further study, however, it became apparent that this formulation of the α-keratin structure was far from satisfactory. The more or less co-planar hexagonal fold postulated by Astbury brought the ketonic carbon and the imino nitrogen atoms of the intra-chain secondary valence bonds (indicated by dotted lines in Figure 4) far too close together for the bonding to be of this type; it was accordingly necessary to assume a covalent character for this linkage. Following the suggestion of Frank[36] this was incorporated in his structure by Astbury,[3] the covalence being accounted for by a lactam-lactim or keto-enol tautomerism along the chain. Other difficulties then arose, however; in addition to the fact that the force necessary to rupture covalent bonds is far greater than the comparatively small force required to effect the α to β transformation, it was difficult to understand why one covalent CONH link should be ruptured in preference to another. The coup de grace was administered to the original Astbury α-keratin formulation by Neurath,[97] who showed by means of scale models that there was simply not space enough in the structure to accommodate the side chains. These are surprisingly bulky, having an average cross-sectional area of about 17 – 25 sq. Å, according to Neurath.

Astbury has accordingly abandoned his original conception of the shape of the α-fold, and Astbury and Bell[7] have recently suggested a modified structure which they claim meets previous objections, and allows sufficient space for

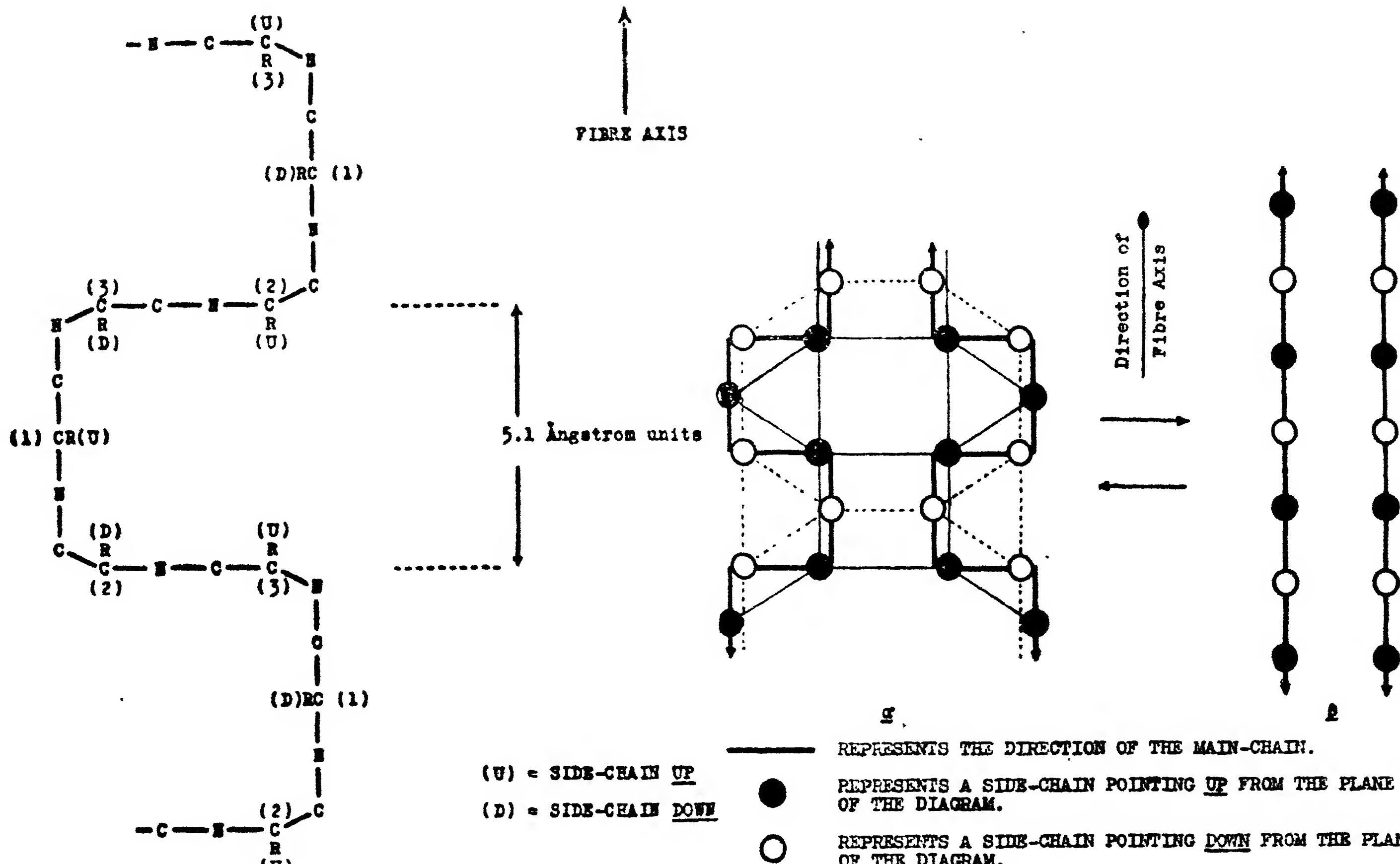


Figure 6. Diagrammatic representation of the structure of α-keratin, according to Astbury and Bell. Left, basis of the intramolecular fold. Right, the close-packing of side chains in the folded structure. From W. T. Astbury and F. O. Bell, *Nature*, 147, 696 (1941), and W. T. Astbury, *Chem. and Ind.*, 60, 491 (1941).

the side chains to project alternately on either side of the fold. This is accomplished by making an approximately rectangular fold, in alternating directions at every third CHR carbon atom. The side chains are thus brought into triangular groups projecting first on one side of the fold and then on the other. Hydrogen bridges are postulated both between opposing CO and NH groups within each fold, and between adjacent folds. The proposed

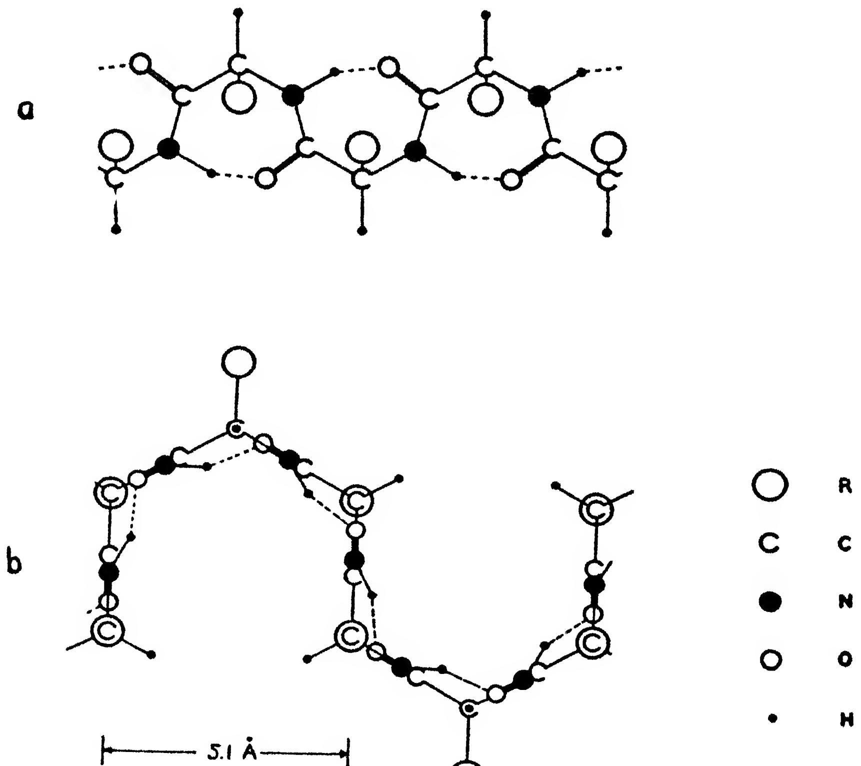


Figure 7. A possible structure for a polypeptide chain in α-keratin, according to Huggins. (a) Idealized ribbon-like structure with intramolecular hydrogen bonds. (b) Edge-on view of (a), showing a possible manner of folding. From M. L. Huggins, *Ann. Rev. Biochem.*, 11, 27 (1942).

structure is shown diagrammatically in Figure 6. Astbury and Bell point out that 5.1 Å is about the shortest distance at which the chain can be folded and still leave the side chains alternately on each side of the fold.

Huggins[58] has very recently critically examined proposed structures for the fibrous proteins, and has pointed out that nearly all of those suggested to date are lacking in probability on the basis of certain well established

structural principles. Thus the most probable structure for a protein would be one in which like groups are all surrounded in a like manner, as far as consistent with differences between the R groups. This principle is not fulfilled by the Astbury and Bell α-keratin structure. Huggins has suggested a modified structure, based on this and other established structural principles, such as that of *close-packing,* which states that the most stable arrangements of molecules are such that each atom or group is surrounded by as many close neighbors as possible, the distances between neighbors being close to the equilibrium distances (*i.e.*, between the forces of attraction and repulsion),

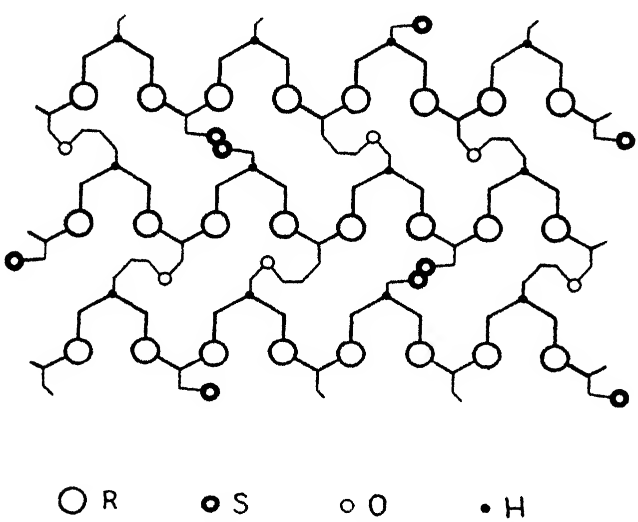


Figure 8 Hypothetical distribution of the spiral chains of Figure 7 in a layer of the α-keratin structure, assuming cystine and serine-glutamate bridges connecting the chains. From M. L. Huggins, *Chem. Rev.*, 32, 195 (1943).

and that there are no large gaps left unoccupied. He has shown that the atoms and bonds of a polypeptide chain may be distributed as shown in Figure 7, by means of intra-chain NHO bridges, to give a ribbon-like structure, which must be folded in order to maintain the proper bond angles. Although there are various ways in which this may be done, Huggins suggests the configuration shown in edge-on view in Figure 7 as the most probable for α-keratin on the basis of the observed x-ray data. It is suggested that the chains are held together in layers (see Figure 8) through cystine bridges and oxygen-containing bridges such as $-CH_2-O-CO-CH_2-CH_2-$, formed by condensation of the side chains of serine and glutamic acid. These layers are stacked together at an average distance of about 9 Å. In the structure proposed, the α- to β-keratin transition does not necessitate the

breaking of any bonds, except the hydrogen bridges. These are changed, by simple shifts of the bridging hydrogens, from intra-chain bonds in the α-keratin to inter-chain bonds in the β-structure. The type of folding suggested, as shown in Figure 7, serves to explain the observed strong x-ray reflection from a spacing of 5.1 Å, without any assumptions regarding differences in the x-ray scattering power among the R groups, such as are necessary with other configurations considered. Huggins, although advocating this type of structure as the most probable on the basis of the observed evidence, emphasizes that the available information is insufficient to permit the deduction of a single unique structure.

The present position with respect to our knowledge of the structure of the keratins might therefore be summarized as follows. It seems reasonably certain that some type of coiled or folded polypeptide chains exists in the α-keratin, and that these are capable of being pulled out almost to the fully extended form, giving the β-keratin, and accounting for the elastic properties. It is not possible to state with any certainty, however, what the exact configuration of the chains is in the α-form. It likewise appears very probable that cystine plays an important role in the structure, probably by tying the chains together into layers through disulfide bridges. As noted in the beginning of this section, the keratins are the proteins of the body's outermost surfaces, where some sort of protective armor is required to meet the contact with the outer world. It seems very likely that nature, in fashioning a suitable material for this purpose, has utilized the properties of the cystine-cysteine oxidation-reduction system discussed earlier. The protein used must be sufficiently labile in its properties to take part in the metabolic processes going on in the tissue where it is formed, and yet, as it is pushed farther and farther out toward the outer surfaces it must become tougher and more resistant to chemical and physical influences. It seems reasonable to suppose that the properties of the cystine-cysteine oxidation-reduction system furnish the needed mechanism, at least in part, for accomplishing this. In the underlying tissues free sulfhydryl groups probably exist in the protein. In the course of its progress toward the outer surfaces, however, more and more of these are oxidized to the disulfide bonds of cystine, thus tying the chains together into the compact, resistant structures which are the keratins of the stratum corneum, nails, or hair.

Collagen

From the technical standpoint, collagen is the most important protein in the skin, because it is present in the largest amount and is responsible for the formation of leather by combination with tanning agents. It is the protein of the white connective tissue fibers of the corium, and constitutes approximately 30-33 per cent of the weight of the whole fresh skin. McLaughlin

and Theis[84] found collagen to compose 84.0, 87.2, and 85.1 per cent of the dry matter of calf, cow, and steer coria, respectively.

Collagen is insoluble in organic solvents, in water, and in dilute solutions of acids and alkalies at ordinary temperatures. According to Nageotte,[94] however, the collagen of the tail tendon of the white rat is soluble in the cold in 0.4 per cent acetic acid, from which it may be precipitated by salting out, or by neutralizing the acid.[117] A distinguishing and highly important characteristic of collagen is its very large swelling capacity in aqueous acid and alkaline systems in the absence of high salt concentrations. These phenomena are considered in detail elsewhere in this book and need not be discussed further here. The prolonged action of hot water converts collagen into gelatin. This reaction, which is of great technical importance, may be considered as the most important single characteristic feature of the protein, aside from its leather-forming and swelling powers. It is, indeed, responsible for the name collagen, which is derived from Greek, and means "glue former." Collagen fibers exhibit a characteristic shrinkage temperature. When heated in water they contract suddenly at a temperature which varies somewhat with the ionic environment and previous treatment, but which is approximately 60-65° C for fibers teased from fresh, untreated skin. Fibers thus contracted are about one-third the original length, have increased considerably in thickness, and possess rubber-like elasticity in the wet condition. This phenomenon will be further discussed in connection with the consideration of the fine structure of collagen.

Pepsin readily digests collagen. Some years ago a considerable controversy existed as to whether or not it is attacked by trypsin. It is now generally considered that native collagen is quite resistant to tryptic action, in the absence of predisposing factors such as swelling, partial degradation by acids or alkalies, etc. Subjection of the material to such conditions, however, rapidly enhances the susceptibility. According to Bergmann[17] fresh collagen fibers are attacked by trypsin only at the cut surfaces.

In the study of collagen two types of preparations have been largely used. In the first of these, where single fibers or fiber bundles are desired, use has been made of tendons (such as the "Achilles tendon") of the larger animals, or the tail tendons of the rat or kangaroo. Although small amounts of other proteins are present in such structures, single fibers or fiber bundles teased from them may be considered to represent essentially collagen. The second type of preparation has resulted from the attempt to purify the original source, usually the derma or corium of skin, from which the non-collagenous constituents are removed by processes designed to affect the collagen as little as possible. In the method described by Highberger[53] this is accomplished by tryptic digestion of fresh corium under carefully controlled condi-

tions, extraction with one-half saturated $Ca(OH)_2$ solution, deliming, and removal of fats with organic solvents. Many variations of this general type of method have been published. It is necessary to point out that the resulting product will fall into one of two different classes, depending upon whether or not a strong alkaline treatment, such as liming to remove the hair, has been used. The official hide powder of the leather chemist, which is made from lime unhaired hides, has an isoelectric point of pH 4.7 – 4.9, and until recently this was supposed to be the isoelectric point of the native collagen. In 1939, however, Highberger[55] and Beek and Sookne[14] simultaneously showed by electrophoretic methods that the isoelectric point of collagen which has not been subjected to a strong alkaline treatment lies at a considerably higher value. The first worker located it at pH 7.8, and the latter investigators at about pH 7.0. The shift in isoelectric point caused by the alkaline treatment is due to some chemical change the exact nature of which is not yet clear, although Beek and Sookne ascribe it to the hydrolysis of side chain amide groups.

The currently generally accepted figures for the amino acid composition of collagen are given in Table 1. Most of the older data related to gelatin — one of the most completely analyzed of the proteins —and only comparatively recently have results based upon collagen preparations of known history become available. Aside from more or less minor variations such as are to be expected in the application of different methods, there is little definite evidence of the existence of collagens of different chemical compositions, such as is found with the keratins. Highberger[54] found the basic amino acid contents of several collagen preparations to be essentially the same. These included preparations from steer, cow, and bull hides, and from different layers of the same steer hide. The anomalous solubility of the collagen of rat-tail tendons may, however, be an indication of a different chemical composition.

According to Grassmann and his co-workers,[46] collagen contains about 0.65 per cent of a carbohydrate, bound as an integral part of the molecule. This will be further discussed in connection with the carbohydrates of skin.

In 1935 Bergmann[15] noted certain regularities in the amino acid composition of gelatin which led him to suggest a skeleton structure for the peptide chains composing this protein, or its precursor, collagen. The principles involved were later elaborated by Bergmann and Niemann[16] into a general theory of protein structure, which has come to be known as the "periodicity hypothesis." The basic postulate of the theory is that the individual amino acids are arranged in the polypeptide chain in a definite pattern such that each individual residue is always separated from a like residue by the same number of other residues. According to Bergmann, this fact forces certain

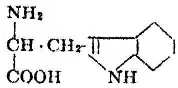
Table 1. Amino Acid Composition of Skin Proteins
(Expressed as per cent on dry protein weight)

Amino Acid	Formula	Keratin (Wool)	Collagen	Elastin
Glycine	NH_2 CH_2 $COOH$	6.5 (20)	26.5 (18)	29.4 (128)
Alanine	NH_2 $CH \cdot CH_3$ $COOH$	4.4 (85)	8.7* (31)	?
Valine	NH_2 $CH \cdot CH(CH_3)_2$ $COOH$	4.72 (85)	0.0* (31)	13.5 (128)
Leucine	NH_2 $CH \cdot CH_2 \cdot CH(CH_3)_2$ $COOH$	11.3[1] (85)	7.1* (31)	30.0[2] (128)
Serine	NH_2 $CH \cdot CH_2OH$ $COOH$	9.41 (86)	4.3[3] (24)	-
Phenylalanine	NH_2 $CH \cdot CH_2 \cdot C_6H_5$ $COOH$	3.75 (85)	1.4* (31)	3.34 (62)
Tyrosine	NH_2 $CH \cdot CH_2 \cdot C_6H_4 \cdot OH$ $COOH$	5.8 (115)	1.0 (40,48)	1.6 (128)
Cystine	NH_2 NH_2 $CH \cdot CH_2 \cdot S \cdot S \cdot CH_2 \cdot CH$ $COOH$ $COOH$	12.5[4] (11)	?	0.23 (128)
Proline	NH—CH_2 CH—CH_2 $>CH_2$ $COOH$	6.75 (85)	17.5 (18)	15.2 (128)

stoichiometric relations upon the molecule, which are discernible in the ratios of the residue fractions of the individual hydrolytic products. Thus the residue fractions* of glycine, proline, and hydroxyproline in gelatin (or collagen) were found to be close to $\frac{1}{3}$, $\frac{1}{6}$, and $\frac{1}{9}$, respectively. Bergmann, therefore, suggested that glycine could be every third amino acid residue in

* These are derived from the percentage figures of Table 1 by dividing by the molecular weight of the respective amino acid, and dividing this quotient (gram moles of the amino acid per 100 grams of protein) by the gram moles of total amino acid residues contained in 100 grams of the protein. The latter value involves an *estimate* of the average molecular weight of all amino acids in the protein, and is consequently a source of some uncertainty.

Table 1. – *Continued*

Amino Acid	Formula	Keratin (Wool)	Collagen	Elastin
Hydroxyproline	NH–CH₂ >CHOH CH–CH₂ COOH	?	14.4* (15)	2.0 (128)
Tryptophane		0.7 (20)	0.0* (34)	0.0 (128)
Arginine	NH_2 $CH\cdot(CH_2)_3\cdot NH\cdot C(:NH)\cdot NH_2$ $COOH$	10.4 (136)	8.4[5] (18)	1.0 (128)
Lysine	NH_2 $CH\cdot(CH_2)_4\cdot NH_2$ $COOH$	3.3 (114)	5.9* (31)	?
Histidine	NH_2 CH–N $CH\cdot CH_2\cdot C$ CH $COOH$ NH	0.7 (20)	0.6[6] (48)	0.0 (128)
Aspartic Acid	NH_2 $CH\cdot CH_2\cdot COOH$ $COOH$	7.27 (124)	3.5 (48)	0.0 (128)
Glutamic Acid	NH_2 $CH\cdot(CH_2)_2\cdot COOH$ $COOH$	15.27 (124)	5.70 (48)	?

Figures in parentheses refer to literature references
* Determined on gelatin [1] Includes isoleucine.
[2] As an impure "leucine fraction" containing also norvaline and phenylalanine
[3] Includes hydroxylysine [4] Values from about 3 to about 15 per cent have been reported for various keratins
[5] The value 9 1 per cent is frequently given for gelatin (75) [6] Dakin (31) reported 0 9 per cent for gelatin

the chains composing gelatin,† proline every sixth, and hydroxyproline every ninth. The two arrangements

(1) – G.P.X.G.X.X.G.P.X.G.X.X.– –
(2) G.X.P.G.X X.G.X.P.G.X.X.– –

where G represents glycine, P proline, and X any other amino acid residue, were suggested as possible structures for gelatin on this basis The isolation by Grassmann and Riederle[47] of the tripeptide lysyl-prolyl-glycine from gelatin would appear to support the second structure. The stoichiometric relations observed among the hydrolytic products of gelatin further led Bergmann to suggest that the precursor of gelatin (collagen) contains a chain of 288 residues, or a whole number multiple thereof, with a minimal molecular weight of 26,000.

The above hypothesis as to the structure of gelatin was based in part on Bergmann's[15] original determination of its proline content as 19.7 per cent.

† This had previously been suggested by Astbury[2] on the basis of his x-ray findings.

This determination was carried out by a maximum precipitation method (with rhodanilic acid), using an empirical correction for solubility.* This figure was later revised by Bergmann and Stein[18] to 17.5 per cent on the basis of determinations made by the "solubility product method" worked out by them. If this value is correct the periodicity, or frequency, of proline in gelatin would be 7 (that is, every seventh amino acid residue along the chain would be proline), which is impossible because of conflict with other frequencies. In a later investigation designed to check the possibility that this value is low owing to racemization of proline during hydrolysis, Stein and Bergmann[127] concluded that 17.5 per cent represents the proline content of collagen and gelatin as determined in the hydrolyzate, but pointed out that the possibility of destruction of proline in the peptide stage is still not excluded. It is apparent that confirmation of this value necessitates modification of the above

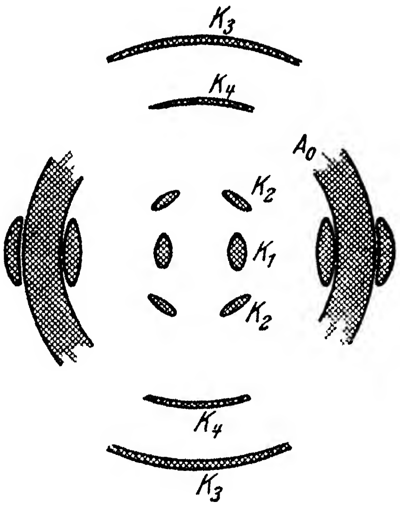


Figure 9. Diagrammatic representation of the x-ray pattern of collagen or of stretched gelatin. Fiber axis vertical. (After J. R. Katz.)

suggested structure, either by abandonment of a structure based on the periodicity principle, or by the adoption of a dual frequency for proline.[18] The possibility must also not be ignored that what we call collagen may be actually a mixture of two or more chemical entities. Some facts emerging from the x-ray investigation also hint at this possibility.

Collagen fibers of diverse origin give similar x-ray patterns which are characteristic and different from those of any of the other fibrous proteins. This pattern is shown diagrammatically in Figure 9. The most apparent reflections are the strong meridional arcs (K_3) of spacing 2.85 Å, the diffuse equatorial reflections (A_0) of about 4.4 Å, and the strong inner equatorial reflections (K_1). A characteristic and striking feature is the variation of the

* Schneider[48] has reported 18.9 per cent proline, determined by rhodanilate precipitation, in cow hide collagen.

value of the latter spacing with the moisture content of the protein. In the dry state it amounts to about 10 Å, and increases with the water content to about 17 Å, beyond which the swollen condition makes observation difficult. Katz and Derksen[64] found the variation in spacing value to be approximately linear with respect to the water content. According to Astbury,[6] the 2.85 Å spacing represents an interplanar distance along the fiber axis which is best interpreted as the length of the amino acid residue; 4.4 Å is the backbone spacing typical of the proteins, and the variable 10 Å spacing represents the side chain distance, at right angles to the fiber axis. The variation of this spacing with the water content is readily explainable on this basis, as it is to be expected that the main polypeptide chains would be pushed farther apart by the entrance into this space of water molecules attracted by the polar groups carried by many of the side chains.

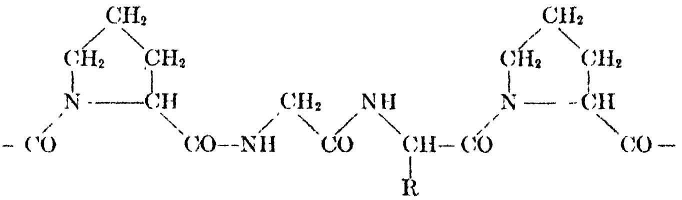
From the few reflections present in these patterns von Susich[129] and also Katz[63] calculated by the method of Polanyi[103] that the period along the fiber axis is 8.4 Å. Astbury[2] concluded that this distance represented, not the length of two amino acid residues, as had been suggested by Meyer,[89] and as does the 7 Å period in silk, but rather the length of three such residues, each of which would then be 2.8 Å in length. According to this interpretation the peptide chains of collagen must therefore involve sequences of three amino acids, and this idea received strong support from the work of Bergmann already discussed. An anomalous situation was presented, however, in the fact that, whereas in all other cases studied, the existence of amino acid residue lengths appreciably shorter than 3.5 Å (the fully extended length) was associated with long-range elasticity in the fiber, this is not true of collagen. Collagen fibers in general have very low elastic limits, and it is apparently not possible to stretch them into the fully extended or β-keratin form.* This anomaly was explained in an ingenious way by Astbury,[6] who has pointed out that the existence of the high proportion of proline and hydroxyproline residues in collagen must exert a large influence on the configuration assumed by the peptide chains, owing to the fact that the nitrogen atom and adjacent α-carbon atom of the five-membered proline ring are both also part of the chain. This limits the rotational possibilities about these atoms, and forces some sort of spiral or folded configuration upon the chain at these points. Using Bergmann's original proline determination, Astbury concluded that every third residue, with the exception of one in every eighteen, is either proline or hydroxyproline, while glycine also occurs as every third residue. The general structure was represented as

—P G—R P—G—R P G—R—

where one glycine and one other residue occur between every two proline (or hydroxyproline) residues. Astbury further concluded that a require-

* See, however, the work of Schmitt, Hall, and Jakus,[117] to be discussed later.

ment of such a structure would be that the side chains must lie in general on the opposite side of the backbone from the proline and hydroxyproline rings (the chains would be in the *cis* configuration) in order to avoid interference. By means of a model of the structure



he showed that a chain meeting these requirements runs on in a straight line, and that the average residue length is 2.85 Å. The only side chains which occur on the same side of the backbone as the proline rings are those of glycine, which are merely hydrogen atoms. The inextensibility of collagen fibers was attributed to the fact that any major alteration in the configuration results in the side chains tending to occupy the same side of the backbone as the proline rings, which is prevented by spatial requirements. Also, in the fully extended condition the chain cannot run on in a straight line, since a sharp change in direction is introduced at each proline residue (see also Pauling[102]), and therefore under these conditions the chain must exist as a series of loops or folds. It must be pointed out that Astbury's suggested structure is not consistent with Bergmann and Stein's later proline determination, discussed previously.

Huggins[58] has recently criticized the Astbury formulation of the collagen structure, and has proposed a somewhat modified structure based on the principles already mentioned in connection with his suggested keratin structure. According to Huggins, the unbalanced forces existing on opposite sides of such a chain as Astbury's model for collagen would tend to bend it continuously in the same direction. He proposes instead a spiral configuration with an average residue length of 2.9 Å, in which the side chains project alternately above and below the main chain. A group of such spiral chains is held together in a layer, as shown in Figure 10, by means of NHO and CHO bridges between different chains. Such layers can be piled together, as indicated in Figure 11, so as to give an approximately "close-packed" arrangement of side chains between each pair, without crowding. Huggins states that the large proportion of proline and hydroxyproline residues cannot fit into any of his other proposed structures, such as those of α- or β-keratin, and that this may explain why collagen assumes the type of structure he suggests. On the other hand, he points out that an equally reasonable assumption would be that the collagen type of structure is, in general, the stable one, and that other structures, such as that of α-keratin, are adopted

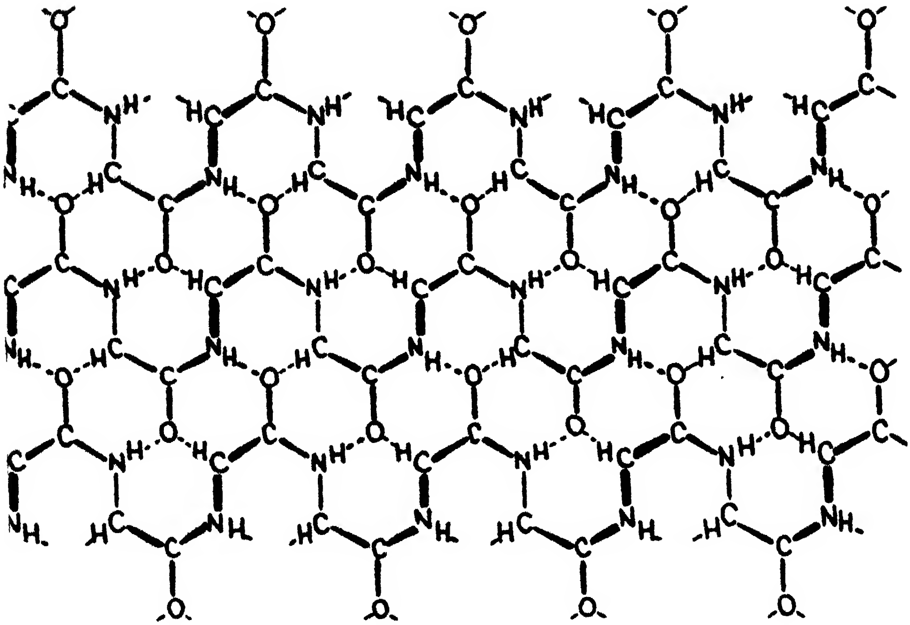


Figure 10 Hypothetical structure of a layer of spiral polypeptide chains in collagen, according to Huggins. An R group or H atom (where the residue is glycine) is assumed to be directly over or directly under the C of each CH. From M. L. Huggins, *Chem. Rev.*, **32**, 195 (1943).

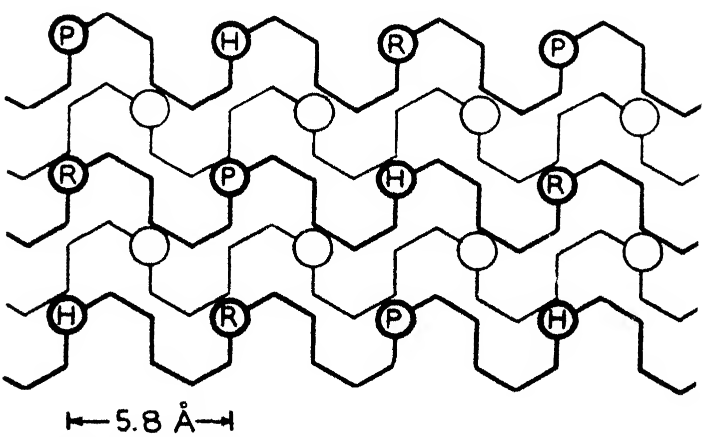


Figure 11. Possible manner of packing adjacent layers of the type of figure 10, in collagen. Heavy circles denote R groups or H atoms pointing *up* from the lower layer; light circles denote R groups or H atoms pointing *down* from the upper layer. The letters P, H, and R indicate a suggested distribution of proline (and hydroxyproline), glycine, and other residues, respectively, on the assumption that these three classes are present in equal numbers. From M. L. Huggins, *Chem. Rev.*, **32**, 195 (1943).

only when there are available a sufficient number of residues, like cystine, capable of bridging between adjacent chains.

The structures proposed by Astbury and by Huggins are based on the existence of a period along the fiber axis which is a multiple of a shortened amino acid residue length of about 2.8 Å, and which Astbury has taken as three times this value, or 8.4 Å. There are, however, reflections present in the collagen x-ray pattern which are not readily reconciled with this, and which lead to a different value for the period. The four spots (K_2) grouped around the central spot, just outside the radius of the side chain reflections (see Figure 9) are the chief example of this. These are present on patterns made from collagens of various sources. According to Trillat,[131] Herzog and Jancke,[52] Küntzel and Prakke,[80] and others, these reflections correspond to a fiber period of 9.8 Å. It has been suggested[90] that this period arises from the length of three amino acid residues, of average length about 3.3 Å, or slightly less than the fully extended length. Meyer[90] suggests that the discrepancies between various workers are best resolved by assuming that two different types of crystals are involved in the formation of the collagen lattice. If this view is accepted it might aid in explaining the apparent odd frequency found for proline by Bergmann and Stein. Relatively few of the proline and hydroxyproline residues would be expected to occur in the portion of the structure composed of chains exhibiting the longer average residue length. On this basis the total collagen structure might be supposed to be the resultant of the association of two crystalline types, one of which, containing large amounts of proline and hydroxyproline residues, is built up from spiral or partially folded chains, while the other is composed of nearly fully extended chains. It must be emphasized that this is pure speculation, and that the few reflections present on the x-ray patterns do not permit the calculation of fiber periods with any certainty.

Several investigators, using special x-ray techniques (longer wave length, increased specimen to film distance), have found very long spacings in collagen fibers. These are certainly to be expected; it was pointed out previously that the periods observed by the ordinary technique are in reality only sub- or pseudo-periods within much larger patterns. It is unfortunate that the study of these longer spacings, which may be expected eventually to yield important results, is at present greatly limited by technical difficulties. Wyckoff and Corey[143] observed several spacings, the highest of which was 330 Å, along the fiber axis of kangaroo tail tendon. Clark and his co-workers[29] found a reflection at 440 Å in catgut, and Bear[12] has reported a spacing of 640 Å. The existing data are as yet too fragmentary to permit any interpretation of these spacings, other than a statement of the probability that they arise from pattern repetitions along very long polypeptide chains.

In 1926 Gerngross and Katz[39] discovered that gelatin stretched about 300

per cent yields an x-ray fiber diagram identical with that of oriented collagen fibers. It had been known for some time that gelatin in its ordinary form gives a Debye-Scherrer or powder diagram identical with that of a mass of unoriented collagen fibers. According to Clark,[29] however, the long spacing at 440 Å is not present in gelatin. From the x-ray results it may be inferred that gelatin contains micelles or crystallites similar in structure, and probably identical with, those of collagen, but present in a disoriented form; on stretching, these micelles become oriented parallel to the direction of stretching.

The phenomenon of the thermal contraction of collagen fibers has been the subject of many investigations. In the shrunken condition the characteristic x-ray pattern of collagen is completely absent, and is replaced instead by an amorphous halo. The optical anisotropy of the fibers is also lost. It is possible, however, to restore the original collagen diagram by stretching the fiber back to its original length.[6] The shrinking has generally been interpreted[6,80] as the result of the folding or coiling of the polypeptide chains, induced by the rupture of linkages holding them in a more extended configuration, and the x-ray and optical data, together with the existence of long-range elasticity in the contracted state leave little doubt of the correctness of this viewpoint. As Astbury[6] points out, however, the fact that the original x-ray pattern can be so readily restored (partially even by spontaneous re-elongation on standing in cold water) can mean only that the general organization of the chains in the micelles remains relatively undisturbed in spite of the folding. The existence in gelatin of micelles which can be oriented to give the typical collagen pattern points to the same conclusion.

It is clear, from the nature of the process, that the thermal contraction of the collagen must be one of the initial phases of the transformation of collagen to gelatin. Cherbuliez and Meyer[28] consider it to be the first, reversible stage of a two-stage process, and regard it as a type of melting. Küntzel,[78] however, believes the contraction to be irreversible, and intimately connected with the liberation of bound water from the structure. The view of Cherbuliez and Meyer is probably more nearly correct.

The newly developed electron microscope technique has been applied to the study of collagen fibers with striking results by Schmitt, Hall, and Jakus.[117] They found collagen preparations from various sources (including human and cattle skin) to be composed primarily of fibrils having widths of the order of 500 to 1000 Å, and exhibiting pronounced cleavage planes parallel to the long axes. Although widths of the above magnitude predominated in the preparations examined, fibrils occurred in widths down to the resolving limit (about 50 Å) of the electron microscope, from which it was concluded that the smallest representatives of the structure had not yet been resolved. The most striking observation was that the fibrils in all cases were striated, possessing alternate light and dark bands, due to regions of relatively

low and high density. The distance between bands of like density varied considerably in different fibrils, but an average value of 644 Å was found. This apparently coincides with the 640 Å spacing found by Bear by the long spacing x-ray technique. The dense and transparent bands were found to have lengths of about 440 and 200 Å, respectively.

Very surprisingly, it was found by Schmitt, Hall, and Jakus that the collagen fibrils were capable of great elongation. This was observed in cases where the supporting collodion film was ruptured and peeled back, exerting considerable tension on the fibrils. Measurements made in such cases showed that the spacing between bands of like density underwent an enormous increase, the maximum observed being nearly 6000 Å. It was shown that the transparent bands increased more than the dense bands, suggesting that material was transferred from the latter to the former during stretching. The hypothesis was suggested that the linear elements of the structure are more highly folded in the dense than in the transparent bands.

Schmitt, Hall, and Jakus point out the discrepancy between these extraordinary phenomena and the physical properties of collagen in the form of intact tendon, which possesses only a small extensibility. Although they offer no definite explanation for this difference in behavior, it is suggested that possibly rupture occurs in the tendon, owing to slippage of fibrils of finite length, before sufficient tension is exerted on the fibrils to elongate them. This seems difficult to reconcile with the considerable tensile strength of tendon. An additional curious observation of these workers was that heat-contracted fibrils still showed the characteristic bands with no appreciable change in spacing. It is evident that these interesting findings need confirmation and further extensive investigation before we can hope to understand them fully. It is tempting to speculate, however, on the relation of the banded structure to the possible existence of two crystal systems in collagen, discussed previously. Schmitt, Hall, and Jakus mention the possibility that the amino acid composition may be different in the two regions. It may be pointed out that the band lengths found in the normal state are insufficient to accommodate chains of 288 residues (Bergmann's unit), even on the basis of the shortest residue lengths yet ascribed to collagen.

It is clear that our understanding of the fine structure of collagen and its relation to the physical properties of the fiber is in perhaps the least satisfactory state of any of the important fibrous proteins. We can be reasonably sure that the structure consists of an assemblage of crystallites composed of long polypeptide chains in semi-crystalline array, more or less parallel to the fiber axis, but as to the exact configuration of the chains and the manner of their packing together we are still very much in the dark. It is probable, however, that future work will resolve many of the difficulties and discrep-

ancies which now exist, and lead to a clearer understanding of this important protein.

Reticulin

It has been known for many years that many of the cellular tissues of the body are surrounded and interpenetrated with a fine, filamentous, network which is called reticular tissue. The protein which composes the fibers of this network has been called reticulin. What little is known about the nature and properties of this material is largely the result of histological observation. The tissue is distinguished from collagenous fibers by its different staining reactions, notably its ability to fix colloidal silver—the so-called argyrophylic property—in contrast to collagen. In 1924 Kaye and Jordan Lloyd[67] observed the presence of reticular tissue in the skin, in the form of a fine fibrous network surrounding and binding together the fiber bundles.* This observation has been confirmed by other workers, especially by Turley,[132] Küntzel,[76,77] Leplat,[81] and Roddy and O'Flaherty.[107]

The nature of reticulin and its relation to collagen have been the subject of dispute ever since the original observations were made, and the matter is still not finally settled. Three schools of thought have developed. The first of these holds that reticulin and collagen are two entirely different and chemically unrelated proteins, the second that reticulin represents an early stage ("precollagen") in the formation of collagen, while the third maintains that reticulin and collagen are the same protein, differing only in the physical disposition of the fibers—the so-called "unitary hypothesis."

According to Kaye,[66] reticulin is a protein which differs in chemical behavior from collagen or elastin, being much more resistant to the action of boiling water and chemical reagents such as acids and alkalies in hot dilute or cold concentrated solutions. It is digested by pepsin, but is only slowly attacked by trypsin. The material swells much less than collagen, and the swelling of collagen fiber bundles is restricted by the presence of the surrounding sheath of reticular tissue. Several workers have demonstrated that on strong swelling collagen fiber bundles are restricted in places by bands of reticular tissue which have survived the rupture of the surrounding network.[66,81]

Küntzel,[76,77] while confirming the existence of a fine fibrous network around the collagen fiber bundles, believed these fibers also to consist of collagen. This view is also subscribed to by Nageotte and Guyon,[95,96] who state that the difference in argyrophylic properties between reticulin and collagen is due to the fact that the much finer fibrils of the former are more easily penetrated by the colloidal silver. They were able to show that fine collagenous fibrils,

* This had previously been pointed out by Seymour-Jones,[120] who first spoke of the tissue as "fiber sarcolemma," and later as "areolar tissue."

produced from the soluble collagen of rat-tail tendon by the method of Nageotte,[94] were just as argyrophylic as reticular tissue. Also, according to these workers, there is no basis in embryology or anatomy for the older "precollagen" hypothesis.

From the results of a recent study of the reticular tissue of animal skin, Roddy and O'Flaherty[107] have concluded that the reticular tissue is really a complex between reticulin and other proteins, such as albumins, globulins, and mucoid. According to this view the other proteins may be considered to constitute a sort of ground-substance for the reticular fibers, the whole complex making up the interfibrillary tissue which surrounds and connects the collagen fibers.

According to Leplat,[81] the reticular tissue is present in its full amount at an early stage in the growth of the animal, so that the proportion of reticulin to collagen is higher in young animals than in adults. In any event the amount of reticulin in the skin must be quite small on a weight basis. The insoluble residue remaining after prolonged boiling in water of purified collagen preparations made from fresh steer corium by Highberger's method, which should contain a large part of the reticulin if the properties ascribed to it are correct, amounts to as little as 0.38 per cent of the original dry weight of the collagen.[53] It must be pointed out, however, that such preparations are made from the middle part of the corium of fully grown animals. It seems probable that larger amounts of reticulin may be present, possibly in association with elastin, in the grain layer of the skin[107] and that these two proteins may comprise in part the resistant proteins which Wilson[139] has termed the "proteins of the grain surface."

Elastin

Elastin, the protein of the yellow elastic tissue, occurs only in small amounts in the skin. Of the dry matter of the corium, it makes up from a few hundredths to about 1 per cent. McLaughlin and Theis[84] found 0.05, 0.27, and 0.92 per cent in the coria of calf skin, cow hide, and steer hide, respectively, on the dry basis.* Elastin fibers form a fine network found mostly in the upper parts of the skin. Where occasional blood vessels occur the elastin content is increased, since this protein makes up a considerable proportion of their walls.

As the name implies, fibers of elastin possess rubber-like elasticity. This property is associated in some manner with their moisture content, since the dry fibers are not elastic. Elastin is insoluble in organic and aqueous solvents, and differs from collagen in being highly resistant to the action of boiling water. It is, however, readily susceptible to tryptic digestion, but is appar-

* These figures refer to the middle 80 per cent of the corium thickness, thermostat and flesh layers having been removed.

ently only slowly attacked by pepsin. Elastin is usually prepared for study from the *ligamentum nuchae*, or stretchy neck ligament, of cattle. The material is cut into small pieces, extracted with dilute sodium chloride solution, washed well, and then extracted repeatedly with boiling water to remove collagen. It is then extracted with 1 per cent potassium hydroxide solution, followed by water and acetic acid. After treating with cold 5 per cent hydrochloric acid solution and washing thoroughly in water, it is again extracted with boiling water, and finally with hot alcohol and ether, after which it is air-dried.

The amino acid composition of elastin is given in Table 1, the most reliable values of which are due to the analysis of Stein and Miller.[128] The protein is made up largely of the simpler amino acids, and the deficiencies in those with polar side chains are reflected in the indifferent chemical properties and limited swelling capacity.

Practically nothing is known definitely about the fine structure of elastin. Astbury considers it to be a member of the group he has characterized as existing in the "supercontracted" state, to which also belong heat-contracted collagen and supercontracted keratin. As has been discussed previously, the essential feature of such structures is the existence of the peptide chains in a highly folded condition, which gives rise to the property of long-range elasticity. Elastin fibers in the ordinary condition give an x-ray pattern which contains only the amorphous halos characteristic of the supercontracted state. According to Kolpak,[68] on stretching and drying elastic ligament a pattern of the collagen type is produced, and the absorption of water by the stretched ligament produces an increase in the side chain spacing similar to that observed with collagen.

Astbury[5] originally suggested that elastin is really a member of the collagen group possessing an abnormally low thermal transformation temperature. Later, however, he came to the conclusion that the collagen pattern observed by Kolpak was probably due to collagen present in the ligaments used.[6] It was shown that the collagen diagram was actually present faintly, in an imperfectly oriented form, in the x-ray photographs of unstretched, unpurified ligaments, and that the only effect of stretching was to orient further the pattern already present. Ligaments from which the collagen had been removed by extracting with water failed to give a collagen pattern on stretching, although the amorphous diagram showed signs of incipient orientation. In view of these facts and the wide discrepancy in the amino acid compositions of elastin and collagen, shown by Stein and Miller's analysis, Astbury[6] therefore modified his original view, and has suggested the possibility that elastin represents a modification lying between the collagen and keratin groups.

Elastin fibers are optically isotropic in the unstretched condition; on stretching they exhibit positive double refraction. These facts, together with

the x-ray observations and physical properties, are in accord with the conception of a structure composed of highly folded or coiled polypeptide chains which on stretching unfold to some extent, but tend to return to the original unstressed configuration. The actual details of the structure and the mechanism of the process are still obscure. It is possible that the large proportion of glycine present may be important in connection with the physical properties.[98]

The Glycoprotein of Skin

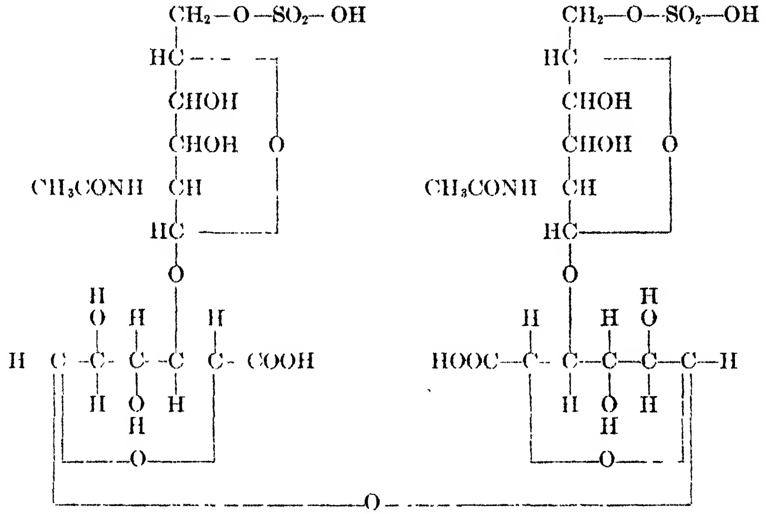
When the corium of skin, carefully freed from soluble components by extraction with sodium chloride solution (or by tryptic digestion under carefully controlled conditions, followed by extensive washing with water), is extracted in the cold with one-half saturated calcium hydroxide solution, a material is dissolved which is precipitated on acidification with acetic acid. This substance has been variously termed the interfibrillary substance, mucoid, coriomucoid, and coriine. When first precipitated it is a cream-colored paste, which rapidly darkens during drying and becomes a brown, horny, brittle mass. McLaughlin and Theis[84] found 0.6, 0.4, and 0.45 per cent in the coria of calf skin, cow hide, and steer hide, respectively, on the dry basis.

The substance contains about 12 per cent nitrogen and about 2.4 per cent sulfur; all of the latter appears to be combined as sulfuric acid.[53] This corresponds to a sulfuric acid content of about 7.3 per cent. Halogens and phosphorus are absent. The Molisch reaction is strongly positive, indicating the presence of a carbohydrate, but Fehling's solution is not reduced until after hydrolysis with hydrochloric acid. Although van Lier[82] obtained a reducing sugar on hydrolysis, Küntzel and Philips[79] were unable to confirm this. According to Grassmann and his co-workers, however, 7.7 per cent galactose plus glucose, and 1.53 per cent of hexoseamine, are present. These results were obtained by spectrophotometric analysis of color reactions produced on the hydrolyzate.

The so-called mucoid of skin was considered by van Lier[82] and others to be the interfibrillary or cementing substance of the connective tissue, and more or less peculiar to this tissue. Abt and Stiasny[1] and Küntzel,[76] however, claimed that of the total lime water-soluble, acetic acid-precipitable material extracted from skin, larger amounts were derived from the epidermal layers than from the corium It is probable that a large part at least of the material considered by these workers to be mucoid was in reality partially degraded keratinous substance, the so-called "keratose." Küntzel believed the mucoid of skin to be identical with cell proteins occurring in protoplasm in general, rather than a specific interfibrillary substance in connective tissue. According to the view of Roddy,[107] the skin mucoid is associated with albumins, globulins, and reticulin in the reticular tissue which invests the collagen fiber

bundles. Whatever the exact nature of its association with other proteins of the skin may be, it seems probable that its function is at least partially that of a protective and lubricating agent for the collagen fiber bundles.

Aside from the few facts already mentioned, little is known of the chemical structure of this substance. By analogy with similar, more thoroughly studied preparations from cartilage, tendon, etc., it is probable that it is composed in part of chondroitin sulfuric acid or a similar sulfuric ester of acetylated hexoseamine and hexoseuronic acid, although aside from the early poorly characterized work of van Lier there appears to be no report extant of the isolation of such an acid from skin mucoid. Considerable confusion exists in the terminology and classification of substances exhibiting both a



Chondroitin Sulfuric Acid

protein and a carbohydrate function, and, although the skin mucoid has generally been classed as a glycoprotein, it is not clear that this is necessarily correct. On the basis of the system of classification recently proposed by Meyer[88] it is probable that the substance would be considered a mucopolysaccharide. Chondroitin sulfuric acid and similar polysaccharide acids are considered to occur in nature either free or as salt-like complexes with a protein moiety. Bungenburgh de Jong,[26] Meyer, and others have shown that these acids form complexes with proteins such as gelatin, which are very similar in properties to the so-called mucoids. The complexes, formed readily by mere mixing of solutions of the respective components, are presumed to be salts formed between the sulfuric and carboxylic acid groups of the polysaccharide unit, and the basic amino groups of the protein.

Although the mucoid occurs in comparatively small amounts in the skin,

its probable importance, both from a technical and a physiological point of view, warrants its further and more detailed investigation.

Albumins and Globulins

The albumins and globulins are the chief representatives of the globular proteins in the skin, from which they may be extracted by dilute sodium chloride solutions. The amounts present, although usually small, vary with the age, sex, and probably with the species. Thus McLaughlin and Theis[84] found 5.1, 1.0, and 1.9 per cent total albumin and globulin in the coria of calf, cow, and steer, respectively, on the dry basis. The higher value observed for calf skin is interesting in view of the fact that these proteins are usually considered to be important food materials for the young animal.

The albumins and globulins are both coagulated by heat. They are both soluble in dilute solutions of neutral salts, but the globulins are insoluble in pure water. This solubility difference affords a ready means of separating the two proteins from a mixed solution in dilute salt solution, since on removing the salt by dialysis the globulin precipitates, while the albumin remains in solution. They may also be roughly separated by various salting out procedures. It is usually stated that the globulins are salted out by half saturation of the solution with ammonium sulfate, whereas the albumins require complete saturation. In the light of modern studies of the physical chemistry of the proteins, however, the results of such separations must be interpreted with caution.

The chief characteristic of these proteins is their great sensitivity to changes in physical and chemical environment, which is reflected not only in their heat coagulability, but also in their response to less drastic influences. They are readily denatured by exposure to light, the presence of certain ions, organic reagents, and even by mechanical influences. The process of denaturation is not well understood, but in general it results in decreased solubility, and is thought to involve fundamental structural changes in the organization of the protein molecules. Astbury[8] has advanced the view, based on x-ray evidence, that denaturation involves increasing crystallinity and tendency of the protein to assume the fibrous type of structure; in this view the fibrous proteins might be considered to exist in a completely denatured state.

According to Grassmann and his co-workers,[46] the globulin of skin contains 15.45 per cent nitrogen, the albumin 15.04 per cent, while each contains 2.2 per cent of a carbohydrate complex consisting of mannose and galactose. One of the principal differences that has been noted in the chemical composition of the two proteins in general, is that the albumins appear usually to contain no glycine, or only very small amounts, while the globulins usually contain this amino acid.

It is very probable that the albumins and globulins of the skin are closely related to, if not identical with, those occurring in the blood stream, from which they are probably derived. Roddy[106] has studied the distribution of these proteins in calf skin by histological methods, and has found them to be present in larger amounts in the epidermal area than in the corium. According to him, in the epidermal region they are intimately associated with the germinating cells and the erector pili muscles. In the corium they are located in the interfibrillary spaces in conjunction with the fibroblasts in their reticular networks.

Lipids

General Lipid Distribution in the Skin

The total amount of lipid in the skin varies greatly from individual to individual within a given species, and is apparently dependent, among other factors, on age, sex, and the dietary habits of the animal. Urbach[135] found amounts varying from 0.7 to 10 per cent of the total weight of the skins of dogs and of humans. McLaughlin and Theis[84] reported total lipids amounting to 0.76, 0.13, and 0.45 per cent on the fresh corium weight, in the coria of young steer, cow, and calf, respectively, but these values are not to be taken as necessarily representative.

Lipids are readily demonstrable in the skin by histological methods, and according to Koppenhoefer[73] histological observations on their amount and distribution check well with chemical results. It is usually observed in stained sections of skin that a more or less variable, but generally large, proportion of the lipid is contained within fat cells, which are masses of fat surrounded by a reticular envelope and deposited here and there among the fiber bundles. In the corium these occur mostly in the lower parts, near the subcutaneous fatty tissue.[99] Only small amounts of lipid are ordinarily observed in the middle portion of the corium, unless the skin is unusually high in lipid content. The upper parts of the corium, and the epidermal layers, however, are relatively rich in lipids. McLaughlin and Theis[84] found on separating young steer corium into three layers, that the upper (27 per cent of the corium weight) contained 1.94 per cent of extractable lipid, the middle (48 per cent of the weight) 0.28 per cent, and the lower (25 per cent of the weight) 1.03 per cent. The distribution of the lipid between the epidermal system, corium, and subcutaneous fatty tissue of fresh goat and sheep skins is given in Table 2, the data of which are taken from the work of Koppenhoefer.[71,72] In Koppenhoefer's work the term "epidermal system, or division," refers to the portion of the skin from the outermost surface to the base of the deepest hair roots, and containing, in addition to the true epidermal layers, the fine fibers and the wandering cells of the corium minor, and the sebaceous and suderiferous glands.

Table 2. Lipid Distribution in Fresh Goat Skin and Fresh Sheep Skin

	Goat Skin			Sheep Skin		
	Epidermis	Corium	Sub-cutaneous	Epi-dermis	Corium	Sub-cutaneous
% Lipid on dry weight	9.20	2.29	57.1	21.8	31.8	93.0
Lipoid phosphorus, gm/kg dry, lipid-free material	558	148	281	829	393	248
Cholesterol, gm/kg dry, lipid-free material	18.5	2.48	4.34	45.0	5.56	5.54
Cholesterol (ester), gm/kg dry, lipid-free material	14.1	1.16	0.0	15.9	0.63	0.0
Free fatty acid, gm/kg dry, lipid-free material	4.65	1.83	13.9	15.9	7.52	14.5
Wax, gm/kg dry, lipid-free material	19.0	—	—	91.5	-	---
Iodine number of ether soluble lipids	43.7	58.8	57.5			
Triglyceride, gm/kg dry, lipid-free material				—	194.3	824

Koppenhoefer[70] also made a more detailed investigation of the lipid distribution in fresh steer hide, which was divided into six horizontal divisions for separate extraction and fractionation of the contained lipids. The summarized results of this work are shown in Table 3. Here the epidermal horn division comprises the outermost epidermal layers, the true epidermis in the histological sense; the transition division refers to a layer, about one-twelfth to one-thirteenth of the total skin thickness, beginning immediately below the base of the deepest hair follicles. The corium major represents slightly more than one-half the total skin thickness, beginning immediately below the transition division. The corium base comprises the balance of the corium, down to, but not including, the subcutaneous fatty tissue. It is evident from the data of Table 3 that the lipid concentration is greatest in the upper (horny and basal epidermal) and lower (corium base) layers. It is to be noted that, although the corium major division contains the smallest percentage of lipid (with the exception of the transition division), it contributes the largest actual amount to the total lipid, because it represents a much larger proportion of the total weight of the skin.

In 1934 Koppenhoefer and Highberger[74] described a detailed procedure, based on the methods of Bloor,[22] for the extraction and fractionation of the lipids of the skin. The original paper must be consulted for details, but in outline the method consists of extraction of the alcohol dehydrated material with boiling alcohol, recovery of the extracted lipids by distillation of the alcohol *in vacuo* (after first cooling and removing a cold alcohol-insoluble fraction which may separate), extraction of the residue with ethyl ether, and separation of the ether-soluble material into an alcohol-soluble and an alcohol-

insoluble fraction. In this manner a rough separation into two groups is effected, one of which, the alcohol-soluble fraction, contains most of the complex lipids, such as cholesterol and the phospholipids, while the other, insoluble in alcohol, contains most of the triglycerides, or simple fats. The original cold alcohol-insoluble fraction, which is characteristic of the epidermal division,

Table 3. Lipid Distribution in Fresh Steer Hide.

	Epidermal region				Corium region	
	Hair	Horn	Basal	Transition	Corium major	Corium base
Dry material (gm)	93	194	396	228	1839	378
Lipid in dry material (gm)	4.28	16.9	28.8	4.45	45.0	27 0
Lipid in dry material (%)	4.60	8.72	7.28	1.95	2.44	7.15
% of total skin lipid	3.39	13.4	22.8	3.52	35.6	21.4
Cholesterol (gm)	0.62	1.96	3.86	0.50	1.17	0.30
Cholesterol in dry material (%)	0.67	1.01	0.97	0.22	0.063	0.079
Cholesterol of division lipid (%)	14.5	11.6	13.4	11 2	2.60	1.11
Lipoid P (mg)	0.0	61.0	373.0	39.3	72.2	16 1
P in dry material (%)		0.031	0.094	0.017	0.0039	0.0042
Acetone-insoluble phospholipid (gm)	0.0	1.02	7.81	0.38	1.43	0.18
P pptd. as lecithin (%)		41.3	67.0	7.2	68.9	34.5
P pptd. as cephalin (%)		6.2	9.9	18.0	4.2	
Phosphorus-cholesterol ratio		1 :32.2	1 :10.3	1 :12.7	1 :16.3	1 :18.6
Free fatty acid (as stearic acid) (gm)	1.15	4 06	1.93	0.49	1.09	0 56
Free acid in dry material (%)	1.24	2.09	0.49	0.21	0.06	0.15
Free acid of division lipid (%)	26.8	17.7	8.3	11.1	4.0	2.05
Wax fraction (gm)	0.90	4.28	10 1			
Wax in dry material (%)	0.97	2.21	2.55			
Wax of division lipid (%)	21.0	25.3	35.1			
Alcohol-insoluble fraction (gm)		1.53	3.96		34.5	21.3
% of dry material		0 79	1.00		1.88	5.65
% of division lipid		9.0	13.7		77.4	80.7
Alcohol-acetone-soluble fraction (gm)	3.38	9.92	7.07	3.82	8.70	4.92
% of dry material	3.63	5 11	1.78	1.68	0.47	1.30
I No. of ether-soluble lipids	50.5	49.8	63.4	58.4	55.6	54.2
Acetyl value of total fatty acids	40.7	47.0	56 8	37.8	2.4	1.4

consists largely of waxes. The main alcohol-soluble and alcohol-insoluble fractions are each subjected to Bloor's procedure for the separation of the phospholipids, consisting of repeated precipitation from ethereal solution with acetone, and finally with alcohol. In this manner three characteristic phospholipid fractions, together with a fraction representing the balance of the

lipids, are obtained from each of the original roughly separated fractions. Koppenhoefer has applied this general procedure to the study of skin lipids with notable success, and the more important results of his work on steer hide will be discussed in detail under the headings of the important lipid constituents of the skin. Some of the results of his studies of the lipids of goat[71] and sheep skins[72] have already been given in Table 2, and the original papers must be consulted for further details. It will suffice here to say that in general, and with minor variations, the lipid picture for these skins is essentially similar to that discussed below for steer hide.

In their original work on the skin fats, McLaughlin and Theis[84] found that the addition of acetic acid to the solvent (acetone) during the extraction of the corium lipids greatly increased the amount of material extractable. Owing to the presence of additional lipid, as well as nitrogenous material, in these extracts, they concluded that a part of the corium lipids was bound to the proteins, and required hydrolysis in order to permit extraction. Koppenhoefer[70] later found, however, that 98 per cent of the total lipid could be extracted by his procedure, and attributed the results of the former workers to the liberation of mechanically held lipid by the partial hydrolysis It is evident from his results that firmly bound lipids, if they exist at all, must be very small in amount, although the possibility exists of loose, easily dissociated complexes between the lipids and other substances.

Triglycerides

The triglycerides or fats are the esters of the fatty acids with the trihydric alcohol glycerol. They may be either simple, where all three acid radicals are the same, or mixed, where different fatty acid radicals are present in the same molecule. From the work of the last few years it would appear that most of the natural fats are composed largely of the latter type. The glycerides of the higher fatty acids are insoluble in water and soluble in organic solvents such as ether, chloroform, benzene, etc. They are readily soluble in hot alcohol and acetone but usually only slightly soluble in these solvents in the cold.

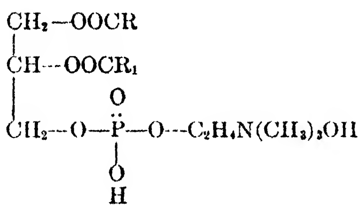
The distribution of the alcohol-insoluble fraction among the various divisions of fresh steer hide is shown in Table 3, from which it is seen to occur predominantly in the corium, where it makes up from 77 to 80 per cent of the total corium lipids, and consists largely of triglycerides. In the epidermal region, on the other hand, triglycerides are found only to a small extent, the main alcohol-insoluble fraction here constituting only from 9 to about 14 per cent of the epidermal lipids, and containing, in addition to triglycerides, large quantities of more complex lipids. Koppenhoefer and Highberger[74] found the amount of the main alcohol-insoluble fraction in the corium to be quite variable from skin to skin, and showed that its analysis was nearly identical

with that of the lipid of the subcutaneous fatty tissue. They concluded from this that the triglycerides of this fraction represented the fat stored in the fat cells of the corium. Application of the lead salt-ether procedure to the corium triglycerides showed that they consisted of about 65 per cent liquid and 35 per cent solid fatty acids. Oleic was found to be the principal liquid acid, and palmitic and stearic the chief solid acids present.

Phospholipids

The phospholipids are substituted fats containing phosphoric acid and a nitrogen base. Three well defined groups are recognized: the lecithins, cephalins, and sphingomyelins. Of these, lecithins and cephalins are always found in the skin, with lecithin in the larger amounts; small percentages of sphingomyelin may or may not be present.[70]

The lecithins and cephalins contain one molecule of glycerol, two of fatty acid, one of phosphoric acid, and one of organic base, which is choline (trimethyl-β-hydroxyethylammonium hydroxide) in the lecithins and amino ethyl alcohol in the cephalins. The structure is usually taken as



for lecithin, where R and R_1 represent fatty acid radicals. Cephalin is supposed to have essentially the same structure, although this is not definitely established, with the substitution of amino ethyl alcohol for choline, and possible differences in the fatty acids. The difference between various lecithins, and between various cephalins, resides largely in the nature and arrangement of the fatty acids present. These may be such acids as stearic, palmitic, oleic, linolenic, linoleic, etc. The lecithins and cephalins usually contain one saturated and one unsaturated acid, although atypical members are known, and according to Koppenhoefer[70] only liquid acids can be separated from the fatty acids of the skin phospholipids by the lead salt-ether procedure.

The lecithins and cephalins are waxy substances which oxidize readily in the air. They are characteristically insoluble in acetone, but soluble in other ordinary fat solvents. Cephalin, however, is insoluble in alcohol, and this property is utilized in its separation from lecithin. Both phospholipids are hygroscopic, miscible with water to form colloidal sols, and capable of acting as emulsifying agents. They are amphoteric, and readily form salt-like combinations with other substances.

Sphingomyelin differs from the lecithins and cephalins in containing no

glycerol, and in the presence of two organic bases, one of which may be either choline or neurine, while the other is sphingosine, an unsaturated hydroxylated amino alcohol. Only one fatty acid radical is supposed to be present, but three acids, lignoceric, cerebronic, and stearic, have been isolated from various preparations. Levene's formula for sphingomyelin is

$$\begin{array}{l} \qquad\quad O{-}C_{17}H_{32}(OH)NH{-}OCC_{23}H_{47} \\ \quad\ \ / \\ O{=}P{-}OH \\ \quad\ \ \backslash \\ \qquad\quad O{-}C_2H_4N(CH_3)_3OH \end{array}$$

In its properties sphingomyelin differs from the lecithins and cephalins chiefly in its greater resistance to oxidation in the air, and in its insolubility in hot or cold ether.

The phospholipids are closely associated with the physiological activity of all living cells, but their exact function is still unknown, although it has been stated to be probably connected with the metabolism of the fatty acids.[23] It seems probable also that their marked stabilizing influence on colloidal emulsions is of importance in the tissues. Koppenhoefer and Highberger[74] found that the complex lipid fraction of the corium bears a constant weight relation to the corium among different skins, and the data of Table 3 show that the distribution of lipoid phosphorus is practically constant throughout the corium. These facts are in accord with what would be expected from the physiological importance of the phospholipids. The lipoid phosphorus reaches a maximum in the basal epidermal layer, which contains nucleated cells and is the site of greatest physiological activity in the skin. The amount decreases in the epidermal horn layer, and falls to zero in the hair. This decrease in phospholipid as the outer surfaces of the skin are approached has been attributed by Kooyman,[69] Koppenhoefer,[70] and others to decomposition accompanying the processes of keratinization.

Table 4, taken from Koppenhoefer's work, gives the analyses of the lecithin fractions isolated from the various divisions of fresh steer hide. The decrease in iodine number and increase in hydroxylation (as indicated by the

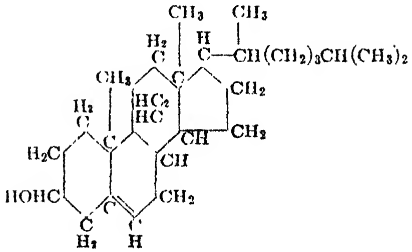
Table 4. Analyses of Lecithin Fractions (Fresh Steer Hide)

	Epidermal region		Corium
	Horn	Basal	major
Lipid (gm)	0.89	6 54	1.29
I No.	60.6	67.7	71.5
% fatty acid	59.7	68.4	61.6
Mean mol. wt.	398.5	360 0	347.0
I No.	66.5	77.2	83.0
Saponification No.	191.5	171.7	183.5
Acetyl value	74.9	52.1	51.2
% unsaponifiable	13.0	8.5	7.0
I No.	45.4	77.1	102.3
% cholesterol	4.4	6.4	1.0
% lipoid phosphorus	2.83	3.82	3.85

acetyl value) as the outer surfaces of the skin are approached are indicative of oxidative degeneration of the phospholipids. The high molecular weights of the fatty acids indicate polymerization or lactone formation, and this also increases toward the outer layers.

Cholesterol

Cholesterol is the characteristic sterol, or solid alcohol, of animal tissues. It is soluble in the ordinary fat solvents, including hot alcohol, but is only sparingly soluble in cold alcohol. Although it is insoluble in water, it has the peculiar property of promoting the absorption of water by fats. Cholesterol is found in all animal cells, particularly in nerve tissues and in the brain. It may occur either free or as an ester in combination with the higher fatty acids. It is related structurally to such physiologically important substances as the bile acids, vitamin D, the sex hormones, and others. In spite of its obvious importance, its physiological function in the tissues is not well understood. According to Windaus,[140] the structure of cholesterol is



Unna and Golodetz[134] found that the cholesterol of skin was associated particularly with the epidermal layers, where together with phospholipids, waxes, and free fatty acids, it makes up a large part of the total lipid. These investigators also observed an increase in cholesterol ester toward the outer surfaces, which they believed to be connected with the processes of keratinization. Similar studies have been made by Eckstein and Wile,[32] and by Kooyman.[69] The distribution of total cholesterol in fresh steer hide is shown in Table 3, from which it is seen that it increases from a small but uniformly distributed amount in the corium to a maximum in the epidermal horn layer. Cholesterol and the phospholipids are probably closely associated in physiological activity. Koppenhoefer[70] found the cholesterol of the corium to exist in the free state, whereas in the epidermis a large proportion is esterified. The cholesterol esters existing in the epidermal layers of the skin are probably derived from the sebaceous secretions, which Koppenhoefer showed to contain cholesterol only in the esterified form. His analysis of the sebaceous secretion (sebum) obtained from fresh steer hide is given in Table 5. The esterification of the cholesterol is probably associated with the break-down

of the phospholipids, part of the fatty acids liberated from the latter combining with the cholesterol to form esters. The cholesterol also undergoes partial oxidation in the outer layers, as is shown by the presence of hydroxycholesterol in the horny, but not in the basal epidermal division.[70]

Roffo[109] has shown that the more exposed parts of the skin contain more cholesterol than the unexposed parts, and that exposure to sunlight increases the cholesterol content.[109, 110]

Waxes

The waxes are esters of the higher fatty acids, in which the alcohol is not glycerol, but is usually a saturated monohydric alcohol or sterol. Waxes may be either liquid or solid. In properties they differ primarily from the fats and oils in their much greater chemical inertness, the source of their value as protective coatings.

Table 5. Analysis of Sebum (Steer Hide).

I No..	32.6
Acid value ..	10.1
Saponification No .	154.8
% fatty acid	57.4
Mean mol. wt	249.1
I No. ..	27.3
Acetyl value ...	74.9
% unsaponifiable .	42.7
I No...	36.0
% total cholesterol.	14.4
% esterified cholesterol	13.7
% lipoid phosphorus	0.159

From Table 3 it may be seen that the waxes of skin are entirely confined to the epidermal region, where they compose from 25 to 35 per cent of the lipid. Table 6, taken from Koppenhoefer,[70] shows the analysis of the wax fractions isolated from the three epidermal divisions. The wax fractions isolated in this manner contain only relatively small amounts of cholesterol esters (but no free cholesterol), the bulk of which occur in the main alcohol-soluble fractions. By comparison of the analyses of these wax fractions with that of the whole sebaceous secretion (see Table 5), which contains considerable quantities of cholesterol esters, Koppenhoefer concluded that two distinct types of waxes are present. One group is composed of esters of higher aliphatic alcohols and saturated hydroxy acids, while the other consists of cholesterol esters in which the acids are more unsaturated. The saturated, oxidized nature of the fatty acids of the first type is evident in the iodine numbers and acetyl values of Table 6; the iodine number of the fatty acids in Table 5 shows the more unsaturated nature of the acids associated with the cholesterol. Koppenhoefer and Highberger[74] isolated isohydroxystearic and stearic acids and arachyl alcohol (*n*-eicosanol) from the epidermal waxes.

Table 6. Analyses of Wax Fractions (Fresh Steer Hide).

	Hair	Epidermal region Horn	Basal
I No.	9.3	4.9	6.5
Saponification No.	149.5	156.0	175.6
Acid value	1.3	0.9	0.3
% fatty acid	75.4	59.2	55.5
Mean mol. wt.	361.0	261.0	252.0
I No.		3.9	5.3
Acetyl value	91.1	85.4	79.2
% unsaponifiable	26.9	44.1	45.4
I No.		6 2	7.4
Acetyl value		182.2	175.0
% cholesterol	7 2	6.2	8.9

According to Koppenhoefer,[70] some lower chain alcohols and acids are also probably present.

The waxes of the epidermal region probably are largely, if not entirely, derived from the sebaceous secretion, in which form they are exuded upon the surface of the skin to form a protective coating. The formation of the cholesterol esters, associated with the hydrolytic and oxidative break-down of the phospholipids, has already been discussed. It is possible that disintegration of the epidermal cells during keratinization may furnish an additional source of waxes.

Free Fatty Acids

In studying the lipids existing on the outer surface of the skin, Kooyman[69] found that they had a high content of free fatty acids. From Table 3 it may be seen that there is a gradual increase in free fatty acid content from the inner to the outer layers of the skin. In the horny epidermal division they comprise nearly 18 per cent of the total lipid, while the lipid of the hair contains about 27 per cent. Kooyman suggested that the high free fatty acid content of the surface lipids was due to the break-down of the lipids by bacteria or lipolytic enzymes.

Koppenhoefer[70] studied the free fatty acids present in the various skin divisions, and found that those of the basal epidermal division varied considerably in character from the combined acids present, showing greater hydroxylation and higher molecular weights than the latter In the epidermal horn division, however, they were similar to the combined acids except for a slightly greater acetyl value, from which it was concluded that some hydroxylation occurred after hydrolysis. The great increase in hydroxylation of the *total* fatty acids as the outer layers of the skin are approached is shown in Table 3.

CARBOHYDRATES

Skin contains only small proportions of carbohydrates. It has been stated that the superficial layers of the skin contain more than the lower.

The total carbohydrate content may be divided into three classes: (1) that intimately related to the blood sugar, and probably derived from it; (2) glycogen; and (3) that bound as an integral part of a protein.

Little if any work has been done on the first two classes in connection with leather-producing skins, and here our knowledge must be derived largely from medical investigations on human skin. Trimble and Carey,[130] from results on a group of diabetic and non-diabetic persons, found that elevation of the blood sugar concentration was accompanied by a marked increase in the sugar content of the skin, while that in the muscle was smaller. In the non-diabetic group the true sugar content (difference between total reducing substances and non-fermentable reducing substances) averaged 56 mg per cent. Folin, Trimble, and Newman[35] had previously shown that intravenous injection of glucose caused almost as high concentration of sugar in the skin as in the blood. They consider that there is a passive diffusion of sugar from blood to skin, and *vice versa*. Rapid elevation of the blood sugar level causes an immediate rapid distribution of sugar into the skin. The process is reversed when the blood sugar level falls below that of the skin, the skin sugar then diffusing back into the blood stream.

Skin contains a small amount of glycogen, the starch-like, polymerized form of glucose used by the body as a reserve store of food. It is claimed that embryonic skin contains larger amounts of glycogen, and that this decreases during fetal life, becoming quite small at birth. Glycogen is said to occur in normal skin in the stratum between the horny (outermost epithelial) and granular layers, in the secretory sweat cells, and in the hair follicle openings. According to Folin, Trimble, and Newman,[35] injection of glucose into the blood stream does not promote glycogen formation in the skin. They found, for instance, 17 mg per cent in normal skin before injection, and 16 mg per cent 2 hours later.

The known sources of bound carbohydrate in the skin have already been indicated to be the proteins collagen, albumins and globulins, and the skin mucoid. The carbohydrates of the latter two have already been discussed in connection with the respective proteins. According to the spectrophotometric results of Grassmann and his co-workers,[46] collagen contains about 0.65 per cent of a disaccharide composed of galactose and glucose. Beek[13] has also studied the carbohydrate of collagen, and has shown by means of a fermentation technique that the collagen hydrolyzate contains no considerable amount of either free *d*-glucose or free *d*-galactose. His spectrophotometric results on the color reactions of a concentrate of the carbohydrate complex were, however, closely similar to those obtained with a mixture of equal quantities of glucose and galactose, from which it was concluded that the carbohydrate either is bound by a difficultly hydrolyzable linkage, or is composed of a mixture of *l*-glucose and *l*-galactose.

Grassmann points out that, although collagen contains the smallest percentage of carbohydrate in comparison to the other skin proteins, it nevertheless accounts for the larger part of the carbohydrate content of the skin, owing to the large proportion of the skin's substance which is made up of this protein.

Mineral Constituents

The principal mineral elements present in the skin are sodium, potassium, calcium, magnesium, and phosphorus. These are probably combined as chlorides, sulfates, carbonates, and phosphates. A portion of the phosphorus is accounted for by the phospholipids. There are practically no data available, however, which permit definite conclusions as to the mode of combination of the mineral elements in general. Most of the data available have been obtained by the analysis of the ash, which of course reveals little on this point. The total mineral matter of the corium of leather-producing skins is usually of the order of 1 per cent, expressed as ash on the dry basis. It is probable that a large part of the mineral content of the skin is derived from salts present in the blood and lymph. Roddy and O'Flaherty[108] have studied the distribution of the mineral matter in fresh calf skin, using a micro-incineration technique. They found the total mineral matter present to be divisible into two fractions, one of which was readily diffusible when the skin was soaked in water before incineration, while the other was not. The latter fraction was small in amount. Both groups appeared to be fairly uniformly distributed throughout both the epidermal and corium areas, being associated with the epidermal cells and with the fibroblasts and fibers in the corium. According to Gans,[38] the potassium of skin is largely localized in the epidermis, whereas most of the calcium is found in the corium.

The analyses of steer, cow, calf, bull, and heifer coria reported by McLaughlin and Theis[84] revealed definite differences ascribable to age and sex.* Calcium and phosphorus were found to be low in the skins of the cow and the heifer, and calf skin contained the highest amounts of magnesium, sulfur, and phosphorus. Additional data are those of Brown[25] on the skins of man, dog, and rabbit,† and those of Loewy and Cronheim,[83] shown in Table 7, on the skins of the rat and the guinea pig. Both these sets of data indicate the extreme variability which occurs in the mineral content of the skin even within a given species. A portion of this variability may be ascribed to variations in the amount of blood and plasma retained within the skin at the time of death, but this seems unlikely to account for the whole of the differences, particularly since pains were taken in most cases to adopt a uniform bleeding procedure in order to avoid this variable.

* The data of McLaughlin and Theis are given as Table III on page 95, Vol. I, second edition of this monograph.

† Brown's data are given in Table IV on page 95 of Vol I.

Table 7. Mineral Constituents of Rat and Guinea-Pig Skin
(Date of Loewy and Cronheim)

	Rat	Guinea Pig
	(Mg per 100 gm dry skin)	
Chlorine	633 - 1441	507 - 1817
Potassium	371 - 446	215 - 244
Calcium	63.7 - 86 6	100
Sodium	212 - 247	252 - 411
Magnesium	20.3 - 27.5	30.3 - 54.5

The small amounts of iron (about 1 mg per 100 gm on the fresh weight) found in the skin are probably to be largely ascribed to the blood hemoglobin, although it is also a constituent of the nuclei and chromatin of cells. Hair also contains iron, which is said to be lowest in the black and highest in the brown varieties. Silica occurs in small quantities, usually in the corium. Brown[25] found the amount in the skin to increase definitely with age. An average of 0.044 gram SiO_2 per kilogram of fat-free skin is reported by Schultz.[118]

Sarata[116] has determined the amounts and distribution of copper in the skins of mottled cats and dogs, with special reference to pigmentation. He found the copper content of dark hair (about 0 6 to 0.7 mg per 100 gm) to be higher than that of colorless hair (0.4 to 0.5 mg per 100 gm). The skin under the pigmented hair showed a higher copper content than that under the colorless. Apparently copper is related to the formation of the melanines.

Zinc is found in small amounts in the skin and hair. The latter is said to contain 9 mg per kg.[112] Eggleton,[33] on the other hand, has reported higher values, ranging from 255 ppm of fat-free, dry hair, to 26 ppm in the skin.

Tin occurs in small amounts in the skin. Values in the range 0.5 to 1 mg per 100 gm have been reported.[133]

Arsenic is said to occur in normal skin to the extent of about 0.026 per cent. The skin and hair, in which it is pathologically deposited, appear to be one of the main pathways of elimination of this toxic element.

Lead is also deposited in the skin and hair in cases of plumbism, although a very small amount is apparently present normally.

Fluorine is said to be present in traces, chiefly in the hair, nails, and epidermis.

Iodine is reported to exist in higher concentrations in the skin than in the blood. The content in normal human skin is from 150 to 200 gamma per 100 gm.[45]

In summarizing the available data on the mineral content of the skin, it is apparent that considerable variation exists, which may be due to age, sex, location on the body, and to individual variation within the species which may be largely a reflection of the diet of the animal.

Water

Like other body tissues, the skin is largely composed of water. The amount present varies somewhat with species, sex, location on the body, and with age and physiological condition, but in general it is between 60 and 70 per cent. McLaughlin and Theis[84] found 61.0 per cent for fresh steer corium, 63.11 per cent for fresh cow corium, and 63.35 per cent for fresh calf corium. It has been stated that in infancy the normal water content of human skin is 81 to 82 per cent, and in old age 72 to 74 per cent, so that there is some evidence of a decrease in water content with age. In cattle skins a similar but less pronounced effect may be seen.

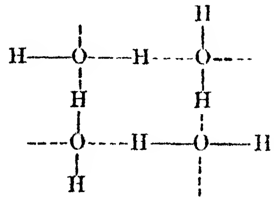
The water-holding capacity of the skin is certainly related to its function as one of the chief temperature regulators of the body. Excess heat produced by vigorous muscular activity must be dissipated, and one of the principal ways in which this is accomplished is by the evaporation of water from the skin surface. It would appear that in general the outer layers of the skin, with the exception of the very outermost, horny layer, contain more water than the inner strata. McLaughlin and Theis[84] found that the top section of whole fresh steer hide, amounting to 20 per cent of the total weight, contained 74.35 per cent water, the middle section, constituting 50 per cent of the weight, contained 61.00 per cent, and the bottom section, 30 per cent of the weight, contained 29.78 per cent. The low value found for the bottom section is probably to be attributed largely to the inclusion of a high proportion of fatty tissues.

The water-holding capacity of the skin is due in large part to the proteins present. The existence of rigid gelatin gels in which the solid material constitutes less than 10 per cent of the total weight is a familiar example of the great ability of the proteins to hold water. The skin, of course, is not a gel, but owing to the relation between collagen and gelatin the forces by which gelatin associates water with itself may be presumed to be similar to those acting in the case of collagen in the skin. The study of such gels, as well as of various animal and plant tissues, has shown that the water in such systems does not display uniform properties. In general, the water in protein-water systems containing 30-35 per cent or less of water behaves differently in many respects from the additional water present at higher moisture values. Hatschek[50] showed, for example, that a gelatin gel containing cobalt chloride showed the pink color of the hydrated salt, but on drying the gel the color changed to the blue of the anhydrous salt at a moisture content of 33 per cent. The water existing in such a system below this critical range, and which exhibits properties different from those of ordinary water, is usually called "bound" water; the remaining water may be called "free" water. Some workers prefer to speak of firmly bound and of loosely bound water. Several

methods depending on the difference in thermodynamic and osmotic properties between these two types have been devised for studying them.

The question arises as to the nature of the forces holding the bound water to the protein. Evidence has accumulated during the past few years which shows that the hydrogen atom can act, under certain conditions, as a bridge or connector between two molecules, or between certain groups in the same large molecule. The resulting bond is called a *hydrogen bond*, or *hydrogen bridge*, and has already been referred to in connection with the structures of the fibrous proteins. Since the hydrogen atom is capable of forming only one normal covalent bond, the hydrogen bond must be of a special type. Study of the conditions under which it is formed has shown that it is largely ionic in type, being formed only between the most electronegative atoms such as fluorine, oxygen, and nitrogen. The capacity of the hydrogen atom to act under these conditions as a bare proton of very small size permits the attracted anions to approach very close to one another. Consequently the formation of the bond will be favored by conditions which promote the ionic character of the bond, such as resonance with structures in which the negativity of the bridged atoms is increased.

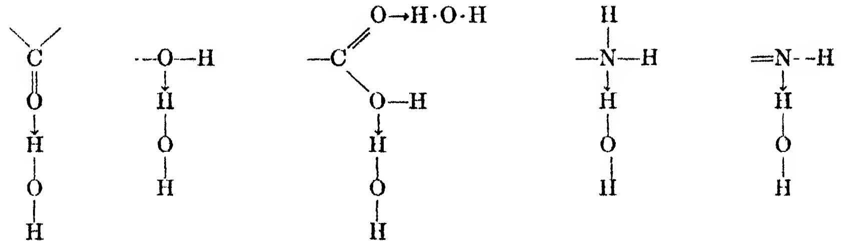
The existence of hydrogen bonds in water itself is largely responsible for the unique physical properties of this substance. It has been stated[101] that if hydrogen bonds did not exist in water its melting and boiling points would be about $-100°$ C and $-80°$ C, respectively. X-ray analysis and other methods of investigation have shown that in ice crystals each oxygen atom is surrounded tetrahedrally by four hydrogen atoms, giving a structure which may be represented as



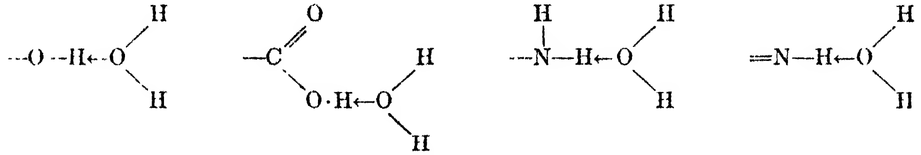
where each hydrogen atom is attracted toward an unshared electron pair of the oxygen atom of a neighboring molecule. It should be noted that the oxygen and hydrogen atoms of the water molecules are not co-linear; the H—O—H valence angle approximates to the tetrahedral angle of about 109°. It has been shown by the study of Raman spectra[30] that in ice at a very low temperature the coordination is nearly complete, with each molecule forming four hydrogen bonds. As the temperature is raised, more and more of the bonds are broken, but a large number still persists in the liquid phase even at the boiling point.

Jordan Lloyd and Phillips[61] pointed out in 1932 that the oxygen and nitro-

gen atoms of proteins presented a number of possibilities for the coordination of water molecules to these structures. Thus it was suggested that the carbonyl, hydroxyl, carboxyl, amino, and imino groups could coordinate water molecules by virtue of the unshared electron pairs on their oxygen or nitrogen atoms:



In addition, the hydrogen atoms of the last four of these groups should be capable of coordinating with the unshared electron pairs of the water oxygen:

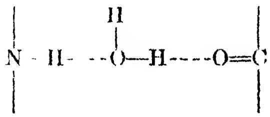


Jordan Lloyd and Phillips suggested that water thus coordinated to the protein structure constitutes the firmly bound water of protein-water systems. The loosely bound water was considered to be held by the orientation of water dipoles around the charged centers existing on the side chains. According to these authors, hydration along the protein backbones will be slight, owing to the presence of direct carbonyl-imino linkages between adjacent chains, which, especially in the fibrous proteins, would reduce the amount of firmly bound water. Jordan Lloyd[59] has calculated, on the basis of one water molecule to each coordination center in the backbones and side chains of gelatin having an assumed molecular weight of 34,500, that about 900 water molecules could coordinate with one molecule of gelatin. This checks reasonably well with the figure of 960 firmly bound water molecules calculated from experimental results on the same molecular weight basis.

Sponsler, Bath, and Ellis[125] have made a more detailed calculation of a similar nature. Assuming a chain length for gelatin of 288 amino acid residues, with a molecular weight of about 27,000, and estimating 450 to 500 water molecules as coordinated along the backbone, they find a total of 800 to 850 bound water molecules per molecule of gelatin. On the basis of the same assumptions the experimentally determined bound water calculates to about 750 water molecules. These authors also point out that it is probable that not all of the possible coordination points along the backbone will be

occupied by water molecules (probably this is especially true of the fibrous proteins), and that consequently several x-ray reflections may be expected from the backbone space, according to whether or not it is interleaved with water molecules. The fusion of these interferences may give rise to the generally poorly defined character of the backbone reflection observed in proteins.

Huggins[57] suggests that the bound water of isoelectric gelatin may be largely held by insertion of the water molecules between the most accessible hydrogen bridges,



and that absorption of additional water involves the formation of water-to-water bridges.

The bound water of tissue probably plays at least two roles. It is known that when proteins lose too much water they become denatured and changed in properties. Proteins, including the fibrous proteins, which have been subjected to drastic desiccation, are unable to rehydrate and swell to the same extent as the normal protein. The bound water may be considered to act as a protection against such denaturation. In addition, it probably also forms a reserve source which may be drawn upon to maintain the necessary free water in the tissue. No hard and fast limits can be drawn between the various types of water; it seems possible that water in several degrees of firmness of binding may be present, and that these are all in a state of dynamic equilibrium with each other and with the free water.

Skin Pigments

The pigments of the skin are apparently to be considered as in the nature of light-absorbing protective filters, although little is known of their real function or chemical structure. The actual chromogenic substances involved in pigmentation are called *melanines*. Although this term has been widely used to include both the chromogenic substances and the conjugated proteins in which they apparently form the prosthetic group, according to Gortner[44] the latter should be called *melanoproteins*. Gortner[43] showed that substances of the latter class could be extracted by dilute alkali from pigmented hair or wool. They are brownish black or black substances which are insoluble in most solvents, including strong acids.

The melanines are formed by the oxidation of dihydroxyphenylalanine ("dopa") by an enzyme in the skin, called dopa oxidase. It occurs in the melanoblasts of the epidermis, and may be demonstrated by soaking skin sections in dilute dopa solutions, when melanine is formed. Albino skins

apparently do not contain dopa oxidase, whereas heavily pigmented skins, such as that of the negro, give strong reactions. It has been stated that ultraviolet light converts tyrosine into dopa, and this, followed by melanine formation through the action of dopa oxidase, may be the mechanism of the darkening of the skin by the prolonged action of strong sunlight.

Enzymes of the Skin

Nearly all the enzymes found in other tissues of the body have also been demonstrated in the skin. This is scarcely surprising, in view of the close connection of enzymes with all living processes. Many of the enzymes, however, occur to a much smaller extent in the skin than in other more physiologically active tissues, as would also be expected. Apparently the first recorded investigations of skin enzymes were those of Sexmith and Petersen,[119] who worked with aqueous suspensions of dried skin powder. Other workers have used cold water extracts of pulped skin, and the juices expressed from skin by means of high pressures.

From the technical point of view probably the most important enzymes in the skin are those concerned with the autolysis, or self-digestion of the tissue. In the absence of bacterial action post-mortem chemical changes occurring in the skin may be presumed to be largely due to the action of these enzymes. The chief enzyme involved in the digestion of the proteins of animal tissue during autolysis is called *cathepsin.* It is a proteinase which hydrolyzes high molecular weight proteins to proteoses and peptones, and also acts on lower peptides. It is inactive in neutral or alkaline solution, requiring a pH of 4 to 5 for its maximum action. Thus the digestion of the tissue during life is prevented by an unfavorable reaction, while after death acidification by the accumulation of lactic and other acids permits the cathepsin to act on the proteins. According to Bergmann and his co-workers,[37] cathepsin is a complex of at least four proteolytic enzymes possessing different specificities.

The negative finding of Wohlgemuth and Yamasaki,[142] who were unable to demonstrate the presence of proteolytic enzymes in adult human skin, although they confirmed the results of others that skin is autolyzable, is attributed by Rothman[113] to the fact that the pH values of their extracts were unsuitable for the enzymatic action. Sexmith and Petersen[119] showed that skin extracts digest casein in weakly acid, but not in weakly alkaline, solution. They found the activity in extracts of adult human and other mammalian skins to be about one-half that of comparable liver extracts (the cathepsin content of the liver is relatively high, among the organs of the body). While adult mammalian skins show considerable autolytic power, the skins of young animals apparently do not undergo autolysis, according to these investigators.

They attribute this to a predominance of synthetic processes in the younger tissue.

According to Blazso,[19] skins from pigmented and unpigmented animals autolyze differently. Unpigmented rabbit skins autolyze at about the same rate at all pH values from 3.8 to 7.3, but skins from pigmented rabbits frequently show a three-fold increase in autolysis at pH 3.8. The same is true of skin from pigmented and unpigmented areas of the same animal. Monacelli[93] has found that granules containing phospholipids appear in human skin during autolysis. The phospholipids are believed to be derived from the decomposition of lipoproteins.

Sexmith and Petersen[119] found that peptidases (which they detected by their action on Witte's peptone) occurred in large amounts in the skins of young animals, but only in traces in adult human and animal skin. Wohlgemuth and Yamasaki[142] have demonstrated in adult skin a dipeptidase which splits glycyl-tryptophane.

Nucleotidase, the enzyme which decomposes nucleic acid to phosphoric acid and nucleosides, is present in the skin, according to Wohlgemuth and Klopstock.[141] Free phosphoric acid is formed in skin extracts during autolysis by the action of this enzyme.

Lipases capable of splitting tributyrin and tristearin were shown to be present in skin by Porter,[104] who also found lecithinases and cholesterol esterase to be present, the latter in large amounts. According to Sexmith and Petersen,[119] the lipases of skin differ from serum lipase in being more active against esters of low molecular weight, such as ethyl butyrate, than against true fats.

Amylase, the starch-splitting enzyme, has been known for a long time to be a normal constituent of the perspiration. Wohlgemuth and Yamasaki[142] found it to occur in large amounts in the skin itself, mostly in the epidermis and in the subcutaneous fatty tissues. Fetal skin is said to contain more than adult skin,[142] and animal skin much more than human.

The presence in skin of glutathione, the tripeptide glutamyl-cysteinyl-glycine, which is intimately involved in the oxidation-reduction systems of the body tissues, has been both affirmed and denied. Kaye[65] found a substance which gave the nitroprusside reaction for free sulfhydryl groups in the cells of the epidermis and the hair follicles. Kaye's results indicated that the substance could be extracted from the tissue with water, and it was accordingly concluded that it was glutathione. According to Walker,[138] however, the substance responsible for the nitroprusside reaction was not extractable by water, alcohol, or ether, and could therefore not be glutathione. The question was later investigated by Giroud and Bulliard,[41] who confirmed Kaye's conclusion, although they found the substance to be extractable by water with difficulty. This was attributed to its being complexly bound in the tissue.

According to Melczer,[87] the hydrogen peroxide-decomposing enzyme, catalase, occurs in all strata of the skin. Various oxidases are also present. Chief among these is that specific for dihydroxyphenylalanine, the so-called dopa oxidase, which has already been discussed in connection with pigment formation.

Space does not permit the discussion of numerous other enzymes which have been found in the skin.

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Chapter 4

Combination of Proteins with Acids, Bases, Salts and Heavy Metals

The protein material of which collagen, sericin, fibroin, hair or wool is composed, contains a number of reactive groups capable of fixing acid or base. Undoubtedly these same reactive groups play an important role not only in combining with acid or base but as well with formaldehyde, vegetable tannins, quinone, chromium, or aluminum and other metallic salts and dyes.

Proteins themselves, as has been already noted, are extremely complex bodies built up of many amino acids bound together through peptide linkage between the α-amino group of one and the α-carboxyl group of another. Proteins are essentially extremely long polypeptide chains. In addition to the elongated chain, the elementary protein fibers or molecules are further linked together through both primary and secondary valency forces. Most proteins yield upon analysis certain amino acids containing more than one carboxylic group, such as aspartic, glutamic and hydroxy-glutamic acids, and certain amino acids containing more than one basic group, such as lysine, arginine and histidine. It is rather self-evident that when these dibasic or dicarboxylic acids are bound into the peptide chain through the carboxylic and amino groups of the α-carbon atom, the elementary protein fiber must then exhibit acid or basic qualities due to the unbound carboxylic or amino groups present in these particular amino acid side chains. Thus in the complex protein we find the elementary fibers bound together longitudinally by primary and secondary valency forces, as described in the previous chapter. In a discussion of the acid or base fixation of proteins we are mainly interested in the available amino and carboxyl groups of the polypeptide chain.

The earliest theories of acid-base fixation by proteins were that of a salt formation, assuming the classic reaction:

$$H_2N{-}R{-}COOH \rightleftharpoons H_2N{-}R{-}COO^- + H^+ \text{ or } R \rightleftharpoons R^- + H^+ \quad (1)$$
$$HOH_3N{-}R{-}COOH \rightleftharpoons {}^+H_3N{-}R{-}COOH + OH^- \text{ or } R \rightleftharpoons R^+ + OH^- \quad (2)$$

the protein acting as a weak base when combining with acids and forming highly ionized salts and, conversely, acting as a weak acid in the presence of bases.

The ionization of a weak acid in aqueous solution may be expressed by

$$(H) \times (A) = k(HA)$$

wherein (HA) constitutes the concentration of the un-ionized acid, (H) and (A) that of the cation and anion into which (HA) dissociates. This expression may then be rewritten

$$(H) = k\frac{(HA)}{(A)}$$

But, when a weak acid is neutralized by a strong base, the concentration of the undissociated acid is very nearly equal to the total acid concentration, and the concentration of the anion is very nearly equal to the salt formed, thus:

$$(H) = k\frac{\text{acid}}{\text{salt}}$$

and at half titration

$$(H) = k\frac{(HA)}{(A)} = 1 \text{ or } (H) = k \text{ or } pk = pH$$

which means in effect that at half titration the hydrogen ion concentration is numerically equal to the ionization constant. Similarly, for the titration of a weak base at the half-titration point, the hydroxyl-ion concentration is numerically equal to the ionization constant of that base.

Referring thus to equations (1) and (2), we can write

$$k_a = \frac{a_{H^+} a_{R^-}}{a_R} \qquad \text{or} \qquad k_b = \frac{a_{R^+} a_{OH^-}}{a_R}$$

where a is the symbol of activity. Such equations were used on the premise that k_a would be given by measurements with alkaline solutions of the amino acid and k_b by those with acid solutions. However values obtained for the amino acids were so small, in the neighborhood of 10^{-9} to 10^{-10} for k_a and 10^{-11} and 10^{-12} for k_b, that they could not be correlated with the k_a for acetic acid or k_b for ammonia. Further, since the amino acids might be regarded as substituted derivatives of acetic acid and ammonia, an acceptance of such values for k_a and k_b implied a drastic influence of the substituent groups on dissociation. Table 8[45] shows the values so obtained for a number of organic acids and certain amino acids.

Table 8. The pk for Organic Acids.

Acid	pk	Amino Acid	pk
Formic	3.69	Glycine	9.75
Acetic	4.74	Alanine	9.70
Propionic	4.80	Valine	9.70
Butyric	4.82	Leucine	9.60
Valeric	4.80	Aspartic	3.7; 9.85
Glutaric	4.30; 4.55	Glutamic	4.2; 9.80

It was this great difference in ionization constants between the organic acids and amino acids that lead Bjerrum[12] in 1920 to formulate the theory that amino acids form zwitterions (dipoles) by simultaneous ionization of both the carboxyl and amino groups, the strength of which might be increased even above that characteristic of the aliphatic acids or amines. Thus Bjerrum was able to point out that the ionization constants so obtained were of an order to be anticipated from the chemical composition. Bjerrum's theory was merely that the titration of amino acid or protein with acid represented the back titration of the charged carboxyl group, and that the titration with base represented back titration of the charged amino group.

Rewriting the classic reaction in terms of the zwitterion concept:

$$^{+}H_3N \cdot R \cdot COOH \rightleftharpoons {}^{+}H_3N \cdot R \cdot COO^- + H^+ \quad \text{or} \quad R^+ \rightleftharpoons R^{\pm} + H^+$$

and

$$H_2O + H_2N \cdot R-COO^- \rightleftharpoons {}^{+}H_3N \cdot R-COO^- + OH^- \quad \text{or} \quad R^- \rightleftharpoons R^{\pm} + OH^-$$

then

$$K_A = \frac{a_{H^+} a_{R^{\pm}}}{a_{R^+}} \qquad \text{and} \qquad K_B = \frac{a_{R^{\pm}} a_{OH^-}}{a_{R^-}}$$

Hitchcock[41] points out that these dissociation equations differ from the classic formulation in that (*a*), the uncharged molecule is written as an amphion, and (*b*), the acidic ionization of the carboxyl group, takes place during the neutralization of an acid solution, while the basic ionization is favored by the neutralization of an alkaline solution which contains the ampholyte anion. Thus by relating a_R and $a_R^{\pm}$ (since each refer to the total electrically neutral ampholyte) and multiplying together $K_A k_b$, we get

$$K_A k_b = a_{H^+} a_{OH^-} = K_B k_a$$

These products are then equal to the ion product of water:

$$K_w = a_{H^+} a_{OH^-} = [H^+][OH^-]\gamma_{H^+}\gamma_{OH^-} \tag{1}$$

The relations then between the various constants are

$$K_A = \frac{K_w}{k_b} \qquad \text{and} \qquad K_B = \frac{K_w}{k_a}$$

The zwitterion system gives much more reasonable values for the constants K_A and K_B: $K_A = 10^{-2}$ to 10^{-3} and $K_B = 10^{-4}$ to 10^{-5}.

The subject of ionization constants of ampholytes is made comparatively simple by using the system adopted by Bronsted:

$$\text{Acid} \rightleftharpoons H^+ + \text{Base}$$

$$NH_4OH \rightleftharpoons NH_4^+ + OH^-, \quad k_b = \frac{a_{NH_4^+} a_{OH^-}}{a_{NH_4OH}}$$

$$NH_4^+ \rightleftharpoons NH_3 + H^+, \quad k_a = \frac{a_{H^+} a_{NH_3}}{a_{NH_4^+}}$$

The application of this system to the dissociation of amino acids is as follows:

$$^{+}H_3N-R-COOH \rightleftharpoons {}^{+}H_3N-R-COO^{-} + H^{+},\quad R^{+} \rightleftharpoons R^{\pm} + H^{+}$$
$$^{+}H_3N-R-COO^{-} \rightleftharpoons H_2N-R-COO^{-} + H^{+},\quad R^{\pm} \rightleftharpoons R^{-} + H^{+}$$

$$K_1 = \frac{a_{H^+} a_{R^\pm}}{a_{R^+}} = \frac{[H^+][R^\pm]\,\gamma_{H^+}\gamma_{R^\pm}}{[R^+]\,\gamma_{R^+}} \tag{2}$$

$$K_2 = \frac{a_{H^+} a_{R^-}}{a_{R^\pm}} = \frac{[H^+][R^-]\,\gamma_{H^+}\gamma_{R^-}}{[R^\pm]\,\gamma_{R^\pm}} \tag{3}$$

Under isoelectric conditions, the concentration of cation and anion should be equal. Thus if equations (2) and (3) are multiplied together, and premising that at the isoelectric point (R^+) and (R^-) are equal, we have

$$K_1K_2 = \frac{a_{H^+} a_{R^-}}{a_{R^+}} = \frac{[H^+]^2[R^-]\,\gamma^2_{H^+}\gamma_{R^-}}{[R^+]\,\gamma_{R^+}}$$

and if I is the value of (H^+) at the isoelectric point, then

$$I^2 = K_1K_2\frac{\gamma_{R^+}}{\gamma_{R^-}\,\gamma^2_{H^+}}$$

and using the Sörensen method of writing hydrogen-ion concentration

$$pI = \tfrac{1}{2}\left(pK_1 + pK_2 - \log\frac{\gamma_{R^+}}{\gamma_{R^-}}\right)$$

where pK_1 and pK_2 are negative logarithms of the acidic ionization constants. By using apparent pK values as determined from pH measurements, we can write

$$pI' = \tfrac{1}{2}(pK_1' + pK_2')$$

In a discussion of the isoelectric point, it must be remembered that this condition may hinge upon the combination of the ampholyte not only with hydrogen or hydroxyl ions but with other anions or cations. Sorensen has defined the isoionic point of an ampholyte as the pH at which it combines equally with acid or base. This value is identical with the isoelectric point only if the ampholyte does not combine with ions other than H^+ and OH^-. Therefore it is in the strict sense only the isoionic point which is determined from pK values of the above equations.

Bjerrum's ideas were expanded by Harris and Harris and Birch,[35] who showed conclusively that the zwitterion concept accounted completely for the titration curves of not only the amino acids but polypeptides and proteins as well. The Harris method of making a titration first in water, then in water-formaldehyde was a unique forward step, since acid groups give the same titration curve in the presence of formaldehyde whereas basic groups do not, thus causing a shift in the alkali curve. By this novel technique, Harris has obtained the ionization constants of many amino acids and polypeptides. Table 9, taken from the work of Jordan-Lloyd,[46] gives the pK values of a number of the amino acids.

Table 9. pK Values for Amino Acids.

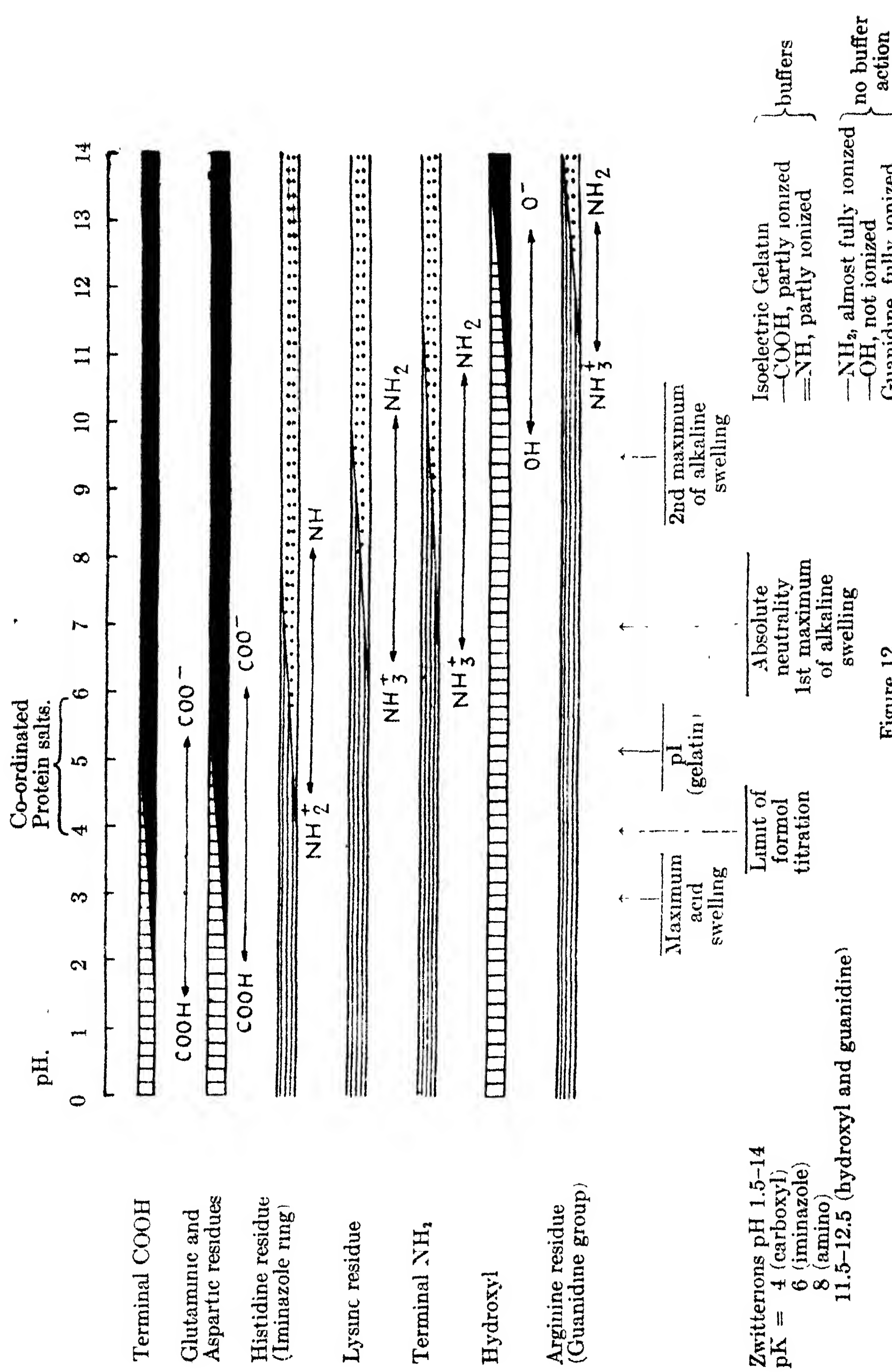
Amino Acid	$t°$	α-COOH	β- or γ- COOH	Imidazole	α-NH₂	OH or SH	ε-NH₂	Guanidine
Glycine	25	2.31	—	—	9.60	—	—	—
Alanine	25	2.21	—	—	9.69	—	—	—
Serine	25	2.11	—	—	9.15	—	—	—
Valine	25	2.20	—	—	9.62	—	—	—
Leucine	25	2.26	—	—	9.61	—	—	—
Iso-leucine	25	2.26	—	—	9.68	—	—	—
Phenylalanine	25	1.50	—	—	9.24	—	—	—
Tyrosine	25	2.24	—	—	9.21	10.28	—	—
Diiodotyrosine	25	2.12	—	—	7.82	6.48	—	—
Tryptophane	25	2.3	—	—	9.39	—	—	—
Cysteine	25	1.60	—	—	7.48	9.02	—	—
Proline	25	1.9	—	—	10.60	—	—	—
Oxyproline	25	1.72	—	—	9.73	—	—	—
Lysine	0	2.20	—	—	9.81	—	11.31	—
Lysine	25	2.18	—	—	8.95	—	10.53	—
Arginine	0	—	—	—	—	—	—	13.31
Arginine	25	1.91	—	—	8.94	—	—	12.48
Arginine	25	2.2	—	—	9.0	—	—	11.9
Histidine	0	—	—	6.50	—	—	—	—
Histidine	25	1.77	—	6.10	9.18	—	—	—
Aspartic acid	25	2.05	3.76	—	9.85	—	—	—
Aspartic acid	25	2.10	3.86	—	9.82	—	—	—
Hydroxyaspartic acid	25	1.95	3.47	—	9.03	—	—	—
Glutamic acid	25	2.00	4.07	—	9.47	—	—	—
Hydroxyglutamic acid	25	2.23	4.24	—	9.56	—	—	—

Jordan-Lloyd goes on to point out that the pK values of both carboxyl and amino groups are influenced by the near presence of other groups attached to the same carbon atom. The influence of different organic groups on the polarities of the COO^- and NH_3^+ groups has been investigated by Phillips[64] and by Melville and Richardson.[58] The recorded pK values for certain amino acids, peptides and amines are given in Table 10.[47]

Table 10. pK Values at 25°

	Carboxyl group	Amino group	Iso-electric point, pI
α-Amino valerianic acid	2.4	9.6	6.0
β-Amino valerianic acid	3.6	10.1	6.9
γ-Amino valerianic acid	4.0	10.3	7.2
δ-Amino valerianic acid	4.2	10.6	7.4
Propionic acid and higher fatty acids	4.8	—	—
Ethylamine	—	10.7	—
Glycine	2.32	9.70	6.06
Glycylglycine	3.03	8.20	5.66
Diglycylglycine	2.90	8.05	5.50
Triglycylglycine	2.95	7.75	5.40
Tetraglycylglycine	2.95	7.70	5.38
Pentaglycylglycine	2.95	7.60	5.32

From a theoretical model of a protein, Figure 12, Jordan-Lloyd[48] postulates that titrations from pH 5.0 on to the acid side will be chiefly titrations of a carboxyl group, and those from pH 5.0 onto the alkaline side will be

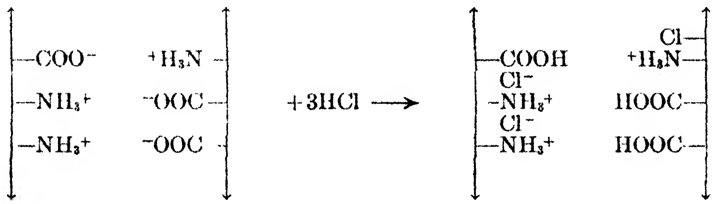


pH.
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14
Co-ordinated Protein salts.
Terminal COOH
COOH
COO⁻
Glutamic and Aspartic residues
COOH
COO⁻
Histidine residue (Iminazole ring)
NH₂⁺
NH
Lysine residue
NH₃⁺
NH₂
Terminal NH₂
NH₃⁺
NH₂
Hydroxyl
OH
O⁻
Arginine residue (Guanidine group)
NH₃⁺
NH₂
Maximum acid swelling
pI (gelatin)
2nd maximum of alkaline swelling
Limit of formol titration
Absolute neutrality 1st maximum of alkaline swelling
Zwitterions pH 1.5–14
pK = 4 (carboxyl)
6 (iminazole)
8 (amino)
11.5–12.5 (hydroxyl and guanidine)
Isoelectric Gelatin
—COOH, partly ionized
=NH, partly ionized
buffers
—NH₂, almost fully ionized
—OH, not ionized
Guanidine, fully ionized
no buffer action

Figure 12

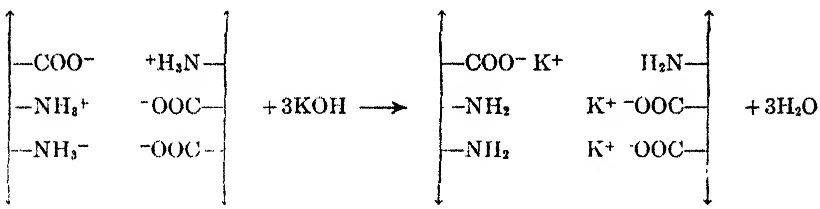
chiefly titrations of, first an imino, secondly an amino, and thirdly a guanidino group.

From such a concept, we may now picture the acid-base binding of a protein:



Thus the protein forms salts by electrovalent links at the amino groups, but the limit of salt formation is primarily controlled by the back titration of the charged carboxyl groups. Phillips[64] postulates that the limit of salt formation is controlled by the intensity of the net charge the protein molecules can accommodate.

In the case of alkali binding the reaction may be illustrated



Jordan-Lloyd[50] points out that in applying the zwitterion theory to the combination of proteins with acids or bases we should have no difficulty in constructing protein salts according to the electronic theory of valence. Maximum salt formation of the type illustrated above will occur at the isoelectric point and therefore there "will not be maximum formation of a fully ionized salt but an equilibrium between ionized and associated base, ionized and associated acid, ionized and associated salt:"

$$R{-}NH_4OH \rightleftharpoons R{-}NH_3^+ + OH^-$$
$$R{-}COOH \rightleftharpoons R{-}COO^- + H^+$$
$$R{-}NH_3 \rightleftharpoons \underset{\underset{O}{\|}}{OC}{-}R \rightleftharpoons R{-}NH_3^+ + {}^-\underset{\underset{O}{\|}}{OC}{-}R$$

History of Protein Titration Curves

As early as 1905 Hardy[34] pointed out that in acid solutions proteins show a positive charge whereas in alkaline solutions they show a negative one. This investigation was in reality the beginning of the study of the acid-base binding power of proteins. Sörensen's classic work in 1909 upon hydrogen ions gave this work an impetus which is felt even today.

As far back as 1898 Bugarsky and Liebermann[17] showed by means of electromotive force measurements that proteins added to themselves acids and bases. For this work, they used methods now long obsolete, but which for their time were far in advance of earlier work. These workers measured the emf between two hydrogen electrodes, one immersed in $0.05N$ hydrochloric-acid solution and the other in the same concentration of this acid, but containing protein. The amount of acid combining was calculated from the well known Nernst equation:

$$E = \frac{RT}{nF} \quad \ln \frac{C_2}{C_1}$$

in which C_2 is concentration of H^+ ions of the solution containing protein and C_1 is concentration of H^+ ions in the $0.05N$ hydrochloric-acid solution.

The classic and usual method of determining the acid-base binding capacity of proteins is indeed a modification of the method used by Bugarsky and Liebermann. Modern potentiometers together with hydrogen or glass electrodes are now generally used; special glass electrodes are available for both the acidic and alkaline range of pH measurement.

Methods for Measurement of Acid-Base Fixation

Measurement of pH value determines the activity of hydrogen ions and not the concentration. For exact calculation, the activity coefficient, α, of the hydrogen ions must be known:

$$\text{pH} = \log \frac{1}{\alpha(C_{H^+})}$$

This activity value is influenced (a) by the concentration of the hydrogen ions, (b) by the concentration of all the ions present, (c) by the valence of the ions, and (d) by the dielectric constant of the solution. In most cases in which the acid-base binding capacity is determined the activities and concentrations are assumed to be the same as for the original acidic or alkaline solutions.

Schmidt[65] points out that due to the assumptions necessary for calculation of H^+ ions bound from activity data, certain deficiencies must be borne in mind when attempts are made to measure activity coefficients of hydrogen ions in protein solutions. If an attempt is made to measure the acid- or base-binding capacity in strong acid or base solutions, the determinations may be in error due to incipient hydrolysis and simplification of the protein, to hydrolysis of the acid amide group, or to the opening up of anhydrides, if present. He also points out the uncertainty of the magnitude of the potential of the liquid junction.

All the calculations from potentiometric data are based upon certain assumptions and are therefore subject to some error. Hoffmann and Gortner,[43] in a study of the prolamines, measured the hydrogen-ion concen-

tration of the acid and alkali solutions before and after the addition of protein and then calculated the degree of ionization of the acid or alkali from the potentiometric data. For such calculation, these investigators used the premise that the determined hydrogen-ion concentration represented a given normality of acid or alkali irrespective of the presence or absence of the protein in the system. For calculation, they used the formula

$$n = N - \frac{(H^+)}{\alpha'}$$

where n is the amount of acid or alkali bound; N is the original normality of the acid or alkali; (H^+) is the hydrogen-ion concentration of the protein-acid or protein-base solution at equilibrium; and α' the degree of ionization of the acid or base as determined by potentiometric methods. Cohn[22] criticized this formula and offered a modification introducing an activity coefficient, γ, in place of the dissociation constant. Gortner then offered criticism of Cohn's suggestion, in that he believed it is necessary to assume that the sodium-protein compound or the protein-chloride compound is completely dissociated; that there is no adsorption of the acid in the molecular state which can give rise to hydrogen ions in solutions; and that the sodium ions from the sodium-protein compound have the same activity as sodium ions in a sodium-hydroxide solution. Other workers have indicated that there may not be complete dissociation of the sodium-protein compound and of the protein-chloride compound. The various detailed methods for determination of the acid-base binding power of proteins by electrochemical means are not given herein, but the reader is referred to the excellent discussion of such methods by Schmidt[66] and by Gortner[28] in their recently published works.

Schmidt has shown that the acid-base combining capacities of proteins as determined by means of classic titration curves involve serious errors. These errors are: (a) the end points are not sharp; (b) the value obtained from a blank titration must be subtracted from the value obtained on titrating the protein; (c) in high acid and alkaline zones, the change in pH value is so small that accurate estimates are almost impossible, and at best the titration represents an equilibrium between the protein salt and the excess H^+ or OH^- ions. Chapman, Greenberg and Schmidt[20] have employed a dye technique for estimating the acid- or base-combining power of proteins. Essentially, the method consists of adding an excess of certain acidic or basic dyes to a protein solution at varying pH values. Upon the addition of the particular dye to the protein solution, a precipitate is formed, which can in turn be removed by filtration. The excess or unused dye can be estimated colorimetrically or by titration with another dye and the combined dye calculated. The data so obtained can be plotted versus pH value as a titration curve. Such data for gelatin have shown 1.03 milliequivalents acid bound per gram

of protein as compared with 0.89 milliequivalent found by Hitchcock[42] by potentiometric methods.

Bancroft[8] applied phase-rule chemistry to this difficult subject. This procedure of determining the acid- or base-combining capacity consists in treating the solid protein with the gas (HCl, H_2S, CO_2 or NH_3) and determining the dissociation pressure. On addition of the gaseous acid or base to the solid protein, the pressure of the gas will remain essentially constant as long as combination takes place. When this reaction is completed there will be a decided increase in the pressure upon further addition of gas. This is in line with the phase-rule relation,

$$F = C - P + 2$$

for in this system there are two components, gas and protein; three phases, solid protein, the protein-gas compound and the gas. There is but one degree of freedom, and since the reaction is carried out at constant temperature, that degree of freedom is destroyed. Upon addition of varying amounts of gas, the pressure must remain constant and will continue so until all the protein has combined with the gaseous acid or base. When this occurs, only two phases are present, the gas and the protein compound. At this point, since temperature is fixed, the pressure rises.

Table 11. Acid- or Base-combining Power of Proteins by Gaseous Titration.

Protein	Acid bound*	Base bound*
Casein	284	212
Gelatin	342	78
Gelatin (deaminized)	305	80
Egg albumin	170	162
Silk fibroin	40	86

*Moles $\times 10^5$ per gram of protein

Czarnetzky and Schmidt[24] made use of gaseous titration. Some of their data are given on Table 11. These investigators show that a difference exists between the groups which combine with HCl or NH_3 when the protein is in the solid state and those which combine with acids and with bases when the protein is in solution. They point out that there should be a difference in acid-combining capacity between a solid protein and gaseous HCl, since more groups of the protein react when in the solid state—the amino groups of lysine, the imino nitrogen of tryptophane (if present), the guanidino group of arginine, the tertiary and imino nitrogen groups of histidine and the tertiary nitrogen of any —CON— groups. They further show some difference in base-combining power, since the hydroxyphenol group of tyrosine does not react with gaseous NH_3. It is concluded that the reactions with gaseous HCl and NH_3 are chemical in nature and, within limits, stoichiometric in value. Beck[9] also used gaseous HCl for his titration values for collagen.

Titration Curves of Amino Acids

Before discussing the titration curves or the acid- and base-binding capacity of proteins, it may be well to study such curves for certain of the amino acids. Greenstein[30] points out that while there have been numerous investigations of the dissociation of amino acids and some simple peptides, little or no data are available relating to the complex peptides involving trivalent amino acids, such as histidine, lysine, arginine, aspartic and glutamic acid, which are able to furnish free polar groups to the protein. He further indicates that any attempt to correlate the acid- or base-combining power of any protein and the strength of apparent dissociation constants with the number of free valencies cannot be successful with data dealing with the simple ampholyte. It would, therefore, seem appropriate to study the manner of behavior of such free groupings, when incorporated in a synthetic peptide having a number of extra valencies; this behavior should be analogous to that of the complex protein.

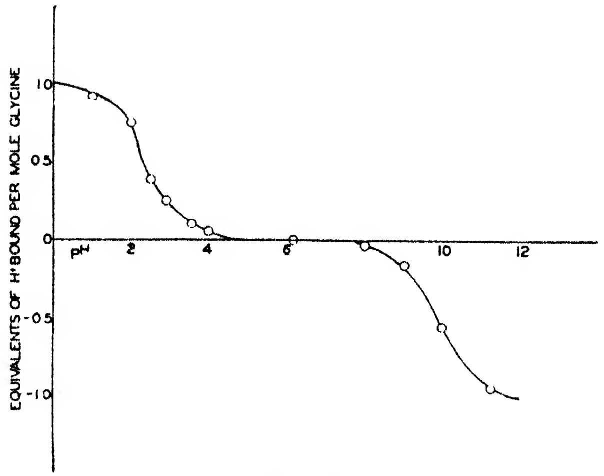


Figure 13

Greenstein studied the dissociation of several of the amino acids, which are able to furnish a free polar group when incorporated into the peptide chain. He measured the dissociation of the amino acid by means of the hydrogen electrode versus the 0.1*N* KCl-calomel electrode. The system was carefully standardized against 0.1*M* HCl. The data and curves taken from the several papers of Greenstein follow.

Figure 13 shows the dissociation curve of glycine at 18° C and ionic strength of 0.1*M*. The points for this curve are taken from the data of Sörensen. This curve shows the maximum base-acid-combining capacity of the

glycine with a pK_1 value of 2.404 and pK_2 value of 9.842. The curve shows a broad buffer region in the pH range 4.0 to 8.0.

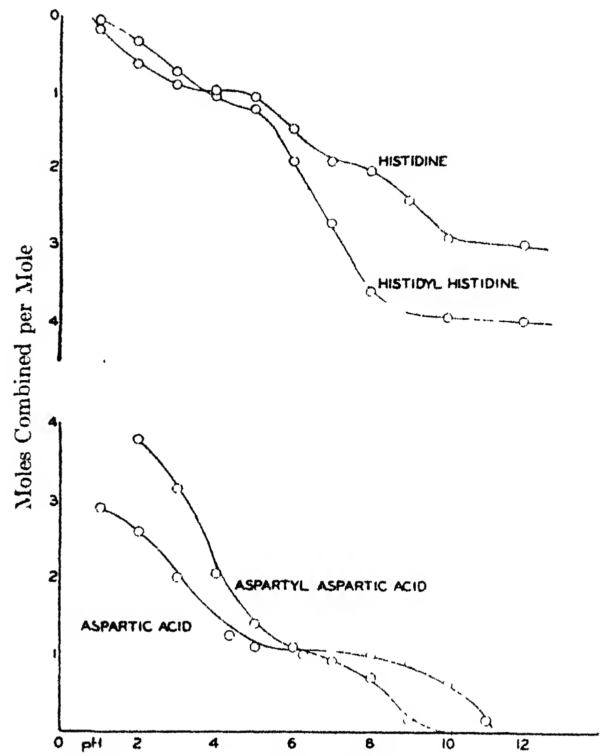
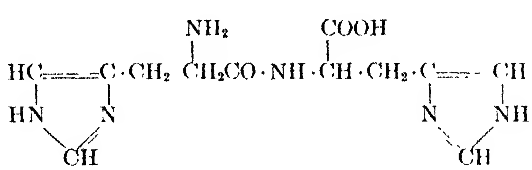


Figure 14

Figure 14 shows Greenstein's curves for histidine, histidyl-histidine and aspartic acid and aspartyl-aspartic acid. Giving histidyl-histidine the structural formula



and aspartyl-aspartic acid the formula

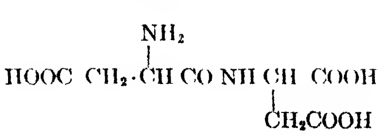


Table 12. Titration Constants

	Carboxyl	Imidazole		Amino	Isoelectric point
Histidine	$\frac{pG_1'}{1.77}$		$\frac{pG_2'}{6.10}$	$\frac{pG_3'}{9.18}$	$\frac{pI}{7.64}$
Histidyl-histidine	$\frac{pG_1'}{2.25}$	$\frac{pG_2'}{5.60}$	$\frac{pG_3'}{6.80}$	$\frac{pG_4'}{7.80}$	$\frac{pI}{7.31}$
	Carboxyl			Amino	Isoelectric point
Aspartic acid	$\frac{pG_1'}{2.10}$		$\frac{pG_2'}{3.86}$	$\frac{pG_3'}{9.82}$	$\frac{pI}{2.98}$
Aspartyl-aspartic acid	$\frac{pG_1'}{2.70}$	$\frac{pG_2'}{3.40}$	$\frac{pG_3'}{4.70}$	$\frac{pG_4'}{8.26}$	$\frac{pI}{3.04}$

we can now study the trends of the curves representing the single basic or acidic amino acid or that representing the amino acids tied into a dipeptide chain. These specific comparative kinds are shown in Table 12.
Greenstein summarizes this work by stating that the introduction of a second imidazole group into the histidine compound causes a decided weakening of the acid and basic groups as compared with histidine itself; that this weakening is accompanied by a shift to the more acid dissociation range of one of the imidazole groups and thus the dipeptide has an isoelectric point more acid than that of the amino acid; that three carboxyl groups to one amino group, as in aspartyl-aspartic acid, result in a weakening of both acid and basic groups and a slightly alkaline shift of the isoelectric point.

In a further study, Greenstein[31] determined the titration constants of tyrosine, glycyl-tyrosine, tyrosyl-tyrosine and diiodotyrosine. The data are given in Table 13.

Table 13. Titration Constants at 25° C.

	Carboxyl	Amino	Hydroxyphenol		Isoelectric point
Tyrosine	$\frac{pG_1'}{2.20}$	$\frac{pG_2'}{9.11}$		$\frac{pG_3'}{10.07}$	$\frac{pI}{5.66}$
Glycyl-tyrosine	$\frac{pG_1'}{2.98}$	$\frac{pG_2'}{8.40}$		$\frac{pG_3'}{10.40}$	$\frac{pI}{5.69}$
Tyrosyl-tyrosine	$\frac{pG_1'}{3.52}$	$\frac{pG_2'}{7.68}$	$\frac{pG_3'}{9.80}$	$\frac{pG_4'}{10.26}$	$\frac{pI}{5.60}$
Diiodo-tyrosine	$\frac{pG_1'}{2.12}$	$\frac{pG_2'}{7.82}$		$\frac{pG_3'}{6.48}$	$\frac{pI}{4.29}$

Thus the data given in Table 13 demonstrate that the assignment of the pH value of approximately 10.0 to the feebly acid oxyphenyl group in tyrosine is consistent with its behavior; that the dissociation of the hydroxyphenyl group depends upon its relative position in the molecule with reference to other free groups; and that the free amino and carboxyl groups in a tyrosine dipeptide are weaker than those of the tyrosine itself. The isoelectric points of the tyrosine peptides are more acid than that of tyrosine

The Titration Curves of the Fibrous Proteins

The work of Greenstein shows that the free polar groups existing in the reactive side chains of the complex polypeptide will exert a definite influence upon the ultimate dissociation constants of the polypeptide. This author points out that while electrostatic forces may account to some extent for the shifting of the values of characteristic groupings in amino acids to the new ranges shown by the proteins, the influence of the peptide link must be taken into account. Thus it may be said that the titration values given for a protein may be related to the ionizable groups of the amino acids not when these acids exist in the free state but only when they are incorporated in a peptide chain.

In the discussion to follow we are much interested in the effect of such free polar groupings of the amino acids, aspartic, glutamic and hydroxy-glutamic acids, histidine, lysine and arginine. The data given in Tables 12 and 13 demonstrate the effect of the imidazole group of histidine and of the second carboxylic group of the acidic amino acids. Undoubtedly the very basic groupings of the arginine and lysine play an important role in determining the various dissociation constants of the proteins of which they are a part.

The acid- or base-binding capacity of such fibrous proteins as gelatin, collagen, silk, hair and wool, have been determined by a great many investigators over a period of years. Atkin and Douglas[4] and Atkin and Campos[5] in 1924 studied the acid-base binding of gelatin and of hide powder. Gortner and Hoffman[43] employing a modified potentiometric method of measuring bound H^+ ion, studied the acid-base binding of the prolamines (vegetable proteins). Hitchcock[42] investigated the acid binding of gelatin and edestin. Manabe and Matula[56] also studied the H^+ and Cl^- binding of protein. In 1935 Jordan-Lloyd and Bidder[40] made quite an extensive study of the H^+ and OH^- binding of hair, gelatin, collagen and silk. In 1940-41, Harris[69] and his co-workers made a thorough and comprehensive investigation of the binding of acid and base of wool and silk proteins. Highberger[38] in 1936 investigated the titration curve of purified collagen. In 1940-41 Theis and Jacoby[70] studied extensively the acid- and base-binding power of collagen, silk and hair. With the exception of Theis and Jacoby, all these investigators used some form of the potentiometric method for measurement of the H^+

or OH^- ion bound by the particular protein studied. Theis and Jacoby[71] used a unique method developed by McLaughlin and Adams.[56] This method and its subsequent modifications to meet specific conditions will be discussed more fully later in this chapter.

The Acid- or Base-binding Capacity of Silk Fibroin

Although the leather chemist is not specifically interested in the fibrous protein silk fibroin, it is illuminating to compare the acid-base-combining power of this fully extended protein with that of the semi-extended proteins such as keratin and collagen.

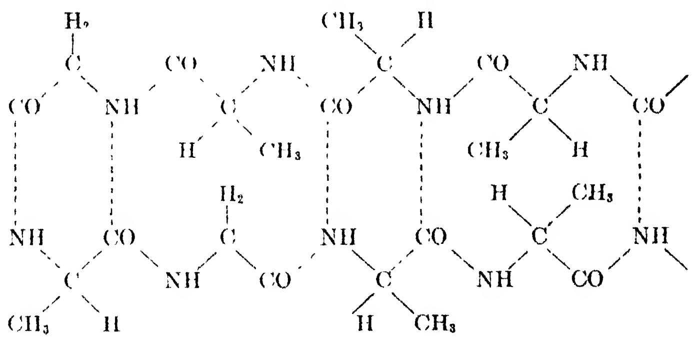
The analysis of silk fibroin indicates that the molecule is made up of a large percentage of glycine and alanine. Table 14 gives the known amino-acid content of fibroin.

Table 14. Amino Acid Content of Fibroin.*

Amino Acid	Percentage	Milliequivalents Per Gram
Glycine	43.8	5.84
Alanine	26.4	2.97
Tyrosine	13.2	0.73
Arginine	0.95	.055
Lysine	0.25	.018
Histidine	0.07	.005

* Taken from the published work of Gleysteen and Harris.

X-ray data and its interpretation indicate that the fibroin structure is one in which the molecules are firmly and tightly held together, in the main the structural cohesion forces being in all probability hydrogen bonds.



Extending from the main axis here and there are a few longer side chains (arginine, lysine, histidine, tyrosine and probably acidic amino acid residues) having free charged groups capable of binding acids or bases.

Jordan-Lloyd and Bidder[49] investigated the acid- and base-binding capacity of gelatin, collagen, silk, hair and wool. These workers found that proteins combine with acids or alkalies on either side of a sharply defined

isoelectric point only if the protein is dispersed as a sol or a gel. They further suggested that an isoelectric zone exists in reality. In a discussion of such data, these investigators have indicated that silk proteins show a sudden take-up of acid or base at pH 3.0 and 11.5, and they believe that such a trend is common to all fibrous proteins, and is accompanied by swelling and disruption of the structure.

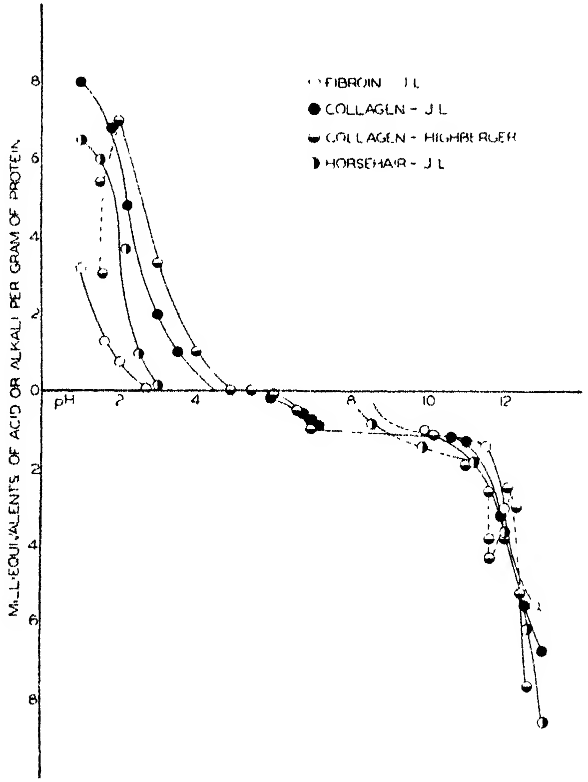


Figure 15

Jordan-Lloyd and Bidder make a comparison between the titration curves of collagen and gelatin and those of keratin and silk; they suggest that the structural cross linkages between peptide chains are somewhat different from the salt linkages and that these bridges exert considerable influence. The titration curves for silk and collagen obtained by these workers are shown in Figure 15.

Gleysteen and Harris[26] recently investigated the acid- and base-binding power of silk fibroin. These workers used a modified potentiometric method for determining the H^+ or OH^- bound and as a consequence were required

to make certain thermodynamic assumptions. They also made corrections for the hydrogen-ion equivalence of the ash and for the sorption of water. They obtained a titration curve at variance with that shown by Jordan-Lloyd and Bidder in that they obtained a definite maximum acid binding at pH = 1.0 of approximately 0.13 millimol per gram. Gleysteen and Harris believe that this discrepancy may be due to the ash content and to incipient hydrolysis of the fibroin. They further suggest that in the alkaline zone,

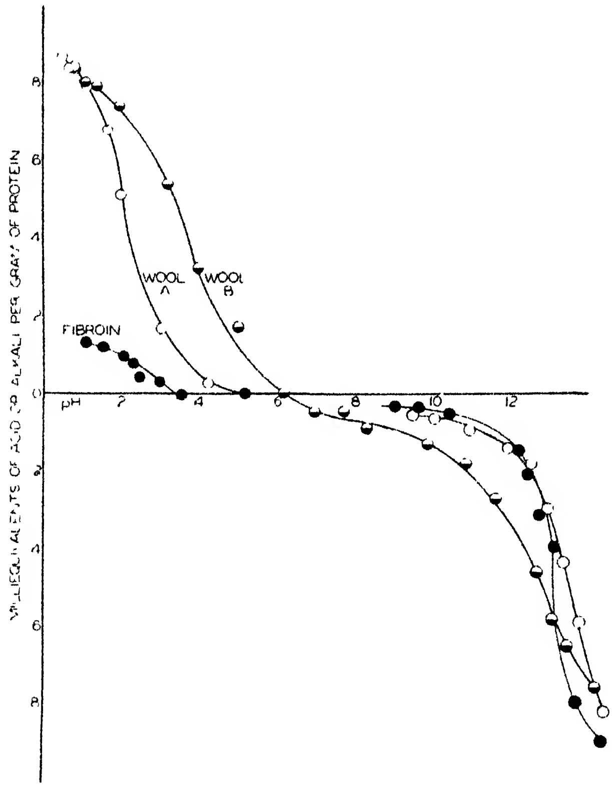


Figure 16

base fixation is partially due to phenolic groups of tyrosine. Figure 16 represents the data taken by these investigators and shows that in aqueous solution of acid or base, no H^+ or OH^- ions are fixed in the pH zone 4.0 to 7.0. However, if salt is present, a somewhat similar curve results, but in the so-called isoelectric zone, slight amounts of acid or base are fixed. Gleysteen and Harris show a maximum acid fixation of some 0.13 millimol at pH = 1.0, and a maximum alkali fixation of approximately 0.9 millimol per gram at pH = 13.8.

Using silk fibroin and natural raw silk, Theis and Jacoby[70] investigated

their acid and base fixation capacity. For this work, they used a rather novel method and obtained titration data quite in accord with those obtained by Gleysteen and Harris.

Before discussing the data obtained by Theis and Jacoby, it might be well to outline briefly the history and development of the titration methods used by these investigators.

McLaughlin and Adams[56] had introduced in 1940 a certain technique of protein-acid analysis. Their method was to treat the protein with acid and after equilibrium had been established, to remove unbound acid by pressing it twice at 5000 pounds per square inch and then analyzing the pressed material for fixed acid. These investigators maintain that this pressing technique removes all the free acid, leaving only that bound or adsorbed by the proteins.

The technique introduced by McLaughlin and Adams was modified and used by Theis and Jacoby. The method so modified gave results well in line with experimental facts The method used was believed free from the errors existing in either the regular or modified potentiometric methods. The method follows.[71]

One-gram portions of the protein material were placed in 200-ml bottles and 100 ml of various concentrations of hydrochloric acid or potassium hydroxide were then added. The concentrations were such that at equilibrium the pH values would vary between pH 0.5 and 13.0. Where neutral salt was used, potassium chloride was employed in the ionic strength noted either in the tables or figures. The bottles and contents were then placed in a thermostat maintained at 20° C for a prescribed period. After equilibrium had been attained, the equilibrium pH value was measured using a Beckman glass-electrode assembly. The treated protein was then pressed several times at 10,000 pounds per square inch in a Carver press. After pressing, the protein was air-dried, ground in a small Wiley mill and was then ready for analysis of H^+, OH^- and nitrogen.

The method for determining H^+ or OH^- ion was as follows. One-half gram of the ground material was carefully weighed into a 250-ml Erlenmeyer flask. To this were added 50 ml distilled water and 10 ml 0.1N HCl. The added HCl aids materially in hydrating the acid-treated protein, and in the case of the alkali-treated protein neutralizes the fixed or bound base and thus converts the protein-base compound into an acid-protein compound. This addition of the HCl is essential for correct results. After a 2-hour treatment as described, 10 ml of a potassium iodine-iodate solution (containing 200 grams KI and 50 grams KIO_3 per liter) are added together with 20 ml of 0.1N sodium thiosulfate and the reagents allowed to react for approximately 2 hours. The excess 0.1N $Na_2S_2O_3$ is then back-titrated with 0.1N HCl or 0.1N iodine solution. A blank determination containing all reagents is run

with each set of experiments. The volume of $0.1N$ $Na_2S_2O_3$ consumed is then calculated to H^+ ion or OH^- ion fixed per gram of protein.

This method is extremely accurate and is comparatively simple. No assumptions need be made and the protein used for the experiment need not be ash, acid- or alkali-free since the method measures only the acid or alkali fixed. This method has many advantages over the potentiometric one since it can be used for the heterogeneous system (fibrous protein-acid), and the amount of acid fixed at any hydrogen-ion concentration is not obtained by a thermodynamic calculation but is an actual quantitative determination upon the protein treated.

Instead of analyzing the ground protein for H^+ or OH^- ion, McLaughlin and Adams[56] determined the anion bound by the protein. This analysis was made by destruction of the protein material by hot nitric acid and then precipitating the anion ($SO_4^=$ in their case) by means of $BaCl_2$. The precipitated $BaSO_4$ was then filtered off, ignited, weighed and calculated to sulfuric acid. In the case of protein treated with hydrochloric acid, the protein is destroyed by means of hot nitric acid in the presence of a definite amount of $0.1N$ $AgNO_3$. After the reaction is complete, the excess $AgNO_3$ is back-titrated with $0.1N$ KCNS, using a ferric salt as an indicator.

The data relating to fibroin and raw silk obtained by Theis and Jacoby are shown in Figure 17. These data indicate a maximum acid fixation at pH 1.0 of 0.16 millimol acid per gram of fibroin. At pH 1.0, curve A of the figure shows a sharp change of slope, really indicative of maximum acid fixation, and the trend of the curve in the pH range 0.5 to 2.0 leaves no doubt concerning a maximum binding of acid in this particular range. These maximum acid-fixation values shown by Gleysteen and Harris and by Theis and Jacoby differ widely from those given by Jordan-Lloyd and Bidder. A study of curve A, Fig. 17, in the pH range greater than 1.0 indicates that acid fixation becomes practically nil at pH 4.0 and remains so until a value of 7.0 is reached. Theis and Jacoby found the alkali fixation in the pH range 7.0 to 11.0 to be slightly different from that obtained by Gleysteen and Harris and they believe such results to be more in line with the facts. Theis and Jacoby in their study find approximately 0.08 millimol alkali bound at pH 11.0 and 0.14 millimol bound at pH 12.0—somewhat lower values than those obtained by other workers. Gleysteen and Harris point out from their data that the dicarboxylic acids of fibroin may be 0.13 to 0.17 milliequivalent per gram as judged by the amount of acid plus base bound between pH 1.0 and 8.0. The data of Theis and Jacoby indicate in this same pH range approximately 0.16 milliequivalent of dicarboxylic acids per gram fibroin. At pH values greater than 11.0, Gleysteen and Harris found that the actual base bound (some 0.9 milliequivalent per gram at pH 13.8) greatly exceeded the content of free carboxyl groups; they accounted for this discrepancy by

postulating the binding of base with the weak phenolic group of tyrosine. Theis and Jacoby found a sharp increase in base fixation in the pH range 7.8 tó 10.0 and another at pH values greater than 11.0. This change in trend of the curve at pH 11.2 must of necessity strongly indicate a change in the base-binding capacity of the protein. At a pH of 12.5 the curve indicates the approach of maximum base fixation, the value being approximately 0.325 milliequivalent per gram of fibroin. This maximum is positive and real. Theis and Jacoby believe that in the pH range 7.8 to 11.0, the basic reaction is with the free carbóxylic groups, the titration of which is complete at pH 11.0. At pH values greater than 11.0, the base in all probability reacts with the weak phenolic groups of tyrosine. The data representing maximum base fixation as given by them is decidedly lower than that given by Gleysteen and Harris—0.325 as compared with 0.90 milliequivalent; but Theis and Jacoby postulate that while some of the weak phenolic groups are reactive, there is not a complete titration of these particular groups. It is quite possible, however, that if Theis and Jacoby had carried titration to approximately pH 14.0, more tyrosine would have reacted; but at this high pH value it was noted that the silk fibroin became "mushy" and could not be pressed and analyzed by their method.

Theis and Jacoby also gave data showing the acid and base fixation of raw silk in contradistinction to that of the fibroin. These curves are represented as B and C in Figure 17. In the acid range pH 1.0 to pH 4.0, Curve B indicates a greater acid fixation, maximum acid fixation at pH 1.0 being 0.35 millimol acid per gram of silk protein. There is also a sharp point of inflection at pH 1.0—again indicative of real values for maximum acid binding. This curve shows a rather broad isoelectric zone pH 4.4 to 5.4. At pH values greater than 5.5, however, base binding begins to increase rapidly to pH 8.0 (degumming taking place due to alkali action upon the sericin of the raw silk). There is another broad zone, pH 8.0 to pH 10.0, beyond which the raw silk binds alkali in much larger amounts as indicated by the increased slope of the curve. We could therefore assume in the pH range of 1.0 to 11.0, we have the combined titration curve of fibroin plus sericin, while at pH values greater than 11.0 only the fibroin is affected, the sericin having been removed and solubilized. Curve B shows a sharp point of inflection in the pH range 10.0-10.5, thus strengthening the points brought out earlier, indicative of tyrosine reaction. At pH 12.0, the curves for raw silk and fibroin merge, both reaching a maximum value at pH 13.0.

Steinhardt and Harris, Gleysteen and Harris, and Theis and Jacoby have shown that the presence of neutral salt causes a greater acid take-up in a certain pH range. Steinhardt and Harris showed that the quantity of acid bound depends upon the concentrations of anions as well as upon the concentration of H^+ ions, and that the quantity of base bound similarly depends upon

the concentration of cations as well as of OH^- ions. They explained this phenomenon on the basis of the assumption that when the protein combines with hydrogen ions it also forms partially dissociated complexes with anions.

Curve C of Figure 17 shows the acid and base titration curve of raw silk in the presence of potassium chloride. In this case acid fixation begins at pH 4.8, and it is shown that there is a sharp isoelectric point in place of the

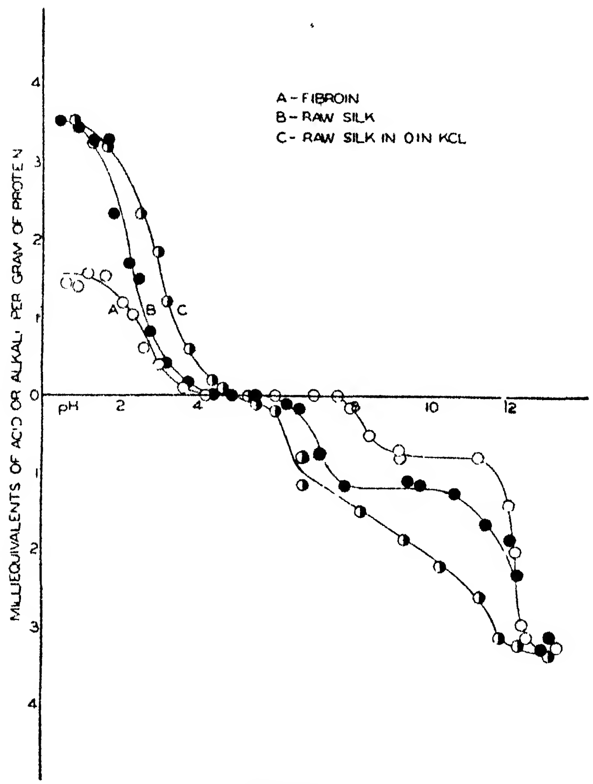


Figure 17

isoelectric zone noticed in curves A and B. As the pH value decreases, acid fixation increases sharply, rising to a real maximum value at pH 1.0 and giving the same value for maximum acid binding as that for raw silk in the absence of salt.

At pH 4.8, the silk begins to fix alkali, the curve representing alkali fixation showing a point of inflection at pH 6.4. At this point the base fixation increases from 0.03 to 0.12 milliequivalent per gram of protein. At pH values greater than 6.3 increased alkali binding results until some 0.34 millimol per gram have been fixed at pH 13.0. At pH values greater than 13.0 this curve

merges with the curves representing raw silk and fibroin in equilibrium with aqueous alkaline solutions.

A comparison of Curves A (fibroin) and B (raw silk) of Figure 17 in the acid range indicates a greater acid binding for raw silk; but in this case it must be remembered that raw silk contains sericin, which in turn contains almost 4 times as much arginine and therefore the maximum acid take-up should be larger.

The Acid-Base-combining Capacity of Hair Keratin

X-ray data have shown that the keratin molecule is semi-contracted, and is capable of both extension and contraction. The molecules of keratin are very compact in nature and are held together in parallel alignment along the axis by hydrogen bonds, by salt linkages between certain charged centers, and by disulfide linkages. It is with the charged centers or free amino and carboxyl groups, the phenolic groups, and possibly the cystine or cysteine groups that acid or base may react. For this reason the content of arginine, lysine, histidine, tyrosine, cystine, glutamic and aspartic acids in the original hair or wool is of real value in estimating the theoretical amounts of acid or base which may react with the keratin fiber. Block[13] in 1938 gave a comparative analysis of various kinds of hair. This analysis dealt only with the basic amino acids. Table 15 shows Block's results.

Table 15. The Basic Amino Acid Content of Hair.

Amino Acid	Lamb Wool	Human	Goat	Camel	Horse[45]
Histidine	0.67	0.60	0.73	0.58	0.60
Lysine	2.50	2 50	3.20	2.70	1.10
Arginine	8.70	8.00	8.10	8.60	7.60
Cystine	11.60	14.70	7.50	8.60	8.00
Tyrosine	4 50	2 90	3 00	3.10	3.20

In a further study in 1931,[14] Block and Vickery showed the basic amino-acid content of various keratins.

Table 16. Basic Amino Acids of Various Keratins.

Protein	Arginine (%)	Histidine (%)	Lysine (%)	Cystine (%)
Human hair	8.0	0.5	2.5	16.5
Sheep wool	7.8	0.66	2.3	10.0
Snake epidermis	5.4	0.48	1.4	5.3
Goose feathers	4.8	0.35	1.04	6.4
Gorgonia flabellum	6.4	0.48	2.75	5.5
Plexaurella dichotoma	5.4	0.43	3.00	3.2
Silk fibroin	0.74	0.077	0.25	0.0

Steinhardt and Harris[69] in 1940 calculated the theoretical acid- and base-binding capacity from the basic and acidic amino acid content of wool.

Table 17. Acidic and Basic Amino Acid Content of Wool and the Theoretical Acid- and Base-binding Capacity.

	Per cent	Millimol per gram
(1) Aspartic acid	7.27	0.545
(2) Glutamic acid	15.27	1.035
(3) Amide nitrogen	1.37	0.978
(1) plus (2) minus (3)		0.602
(4) Arginine	10.2	0.586
(5) Lysine	3.3	.226
(6) Histidine	0.66	.044
(4) plus (5) plus (6)		0.856
(1) plus (2) minus (3) plus (4) plus (5) plus (6)		1.458
(7) Tyrosine	5.8	0.320

Hegman[36] in 1942 gave the basic amino acid content of feather keratin and the calculated theoretical acid- and base-binding capacity.

Table 18. Basic Amino Acid Content of Feather Down

	Per cent	Millimol per gram protein
Arginine	5.72	0.329
Lysine	0.50	0.034
Histidine	0.00	0.000

Jordan-Lloyd and Bidder[49] investigated the acid base combination of horse hair. Their curve showed acid combination at approximately pH 3.0 and a maximum value of about 0.65 milliequivalent per gram at pH 1.0. On the alkaline side of the very broad isoelectric zone, base combination began at pH 8.5 and showed at pH 13.0 a value of 0.83 milliequivalent of base bound per gram. This particular curve showed a very broad isoelectric zone, from pH 3.0 to 8.5. These workers point out in a summary of their paper that a study of the fibrous proteins showed an increasing extension of the isoelectric zone with increasing compactness of fiber structure. In this zone acid or base combination occurs to a very limited extent only. These workers further point out that at pH values less than 3.0 or greater than 11.5, there is a sudden increase in the amount of acid or base bound, and that this is not determined by the amino acid constitution of the protein. They also believe that even in the isoelectric zone the results are influenced also by the structure of the fibers. The data of Jordan Lloyd and Bidder have been given in Figure 15

Steinhardt and Harris[69] made an extensive investigation of the acidic and basic properties of wool. In this study, these workers investigated the presence of neutral salt upon the acid or base fixation. They found that the maximum amount of acid or base bound was independent of the presence or absence of salt, but that the way in which this maximum is approached

depends greatly upon the presence and amount of neutral salt. They also point out that the ability of the protein to combine with H^+ ions is limited by the simultaneous availability of, say, Cl^- ions.

Steinhardt and Harris have shown that the titration curves of wool and a soluble protein in the total absence of salt vary widely. However, in the presence of salt the curves for the two proteins are similar in nature. From these data, Steinhardt and Harris conclude that it should be possible

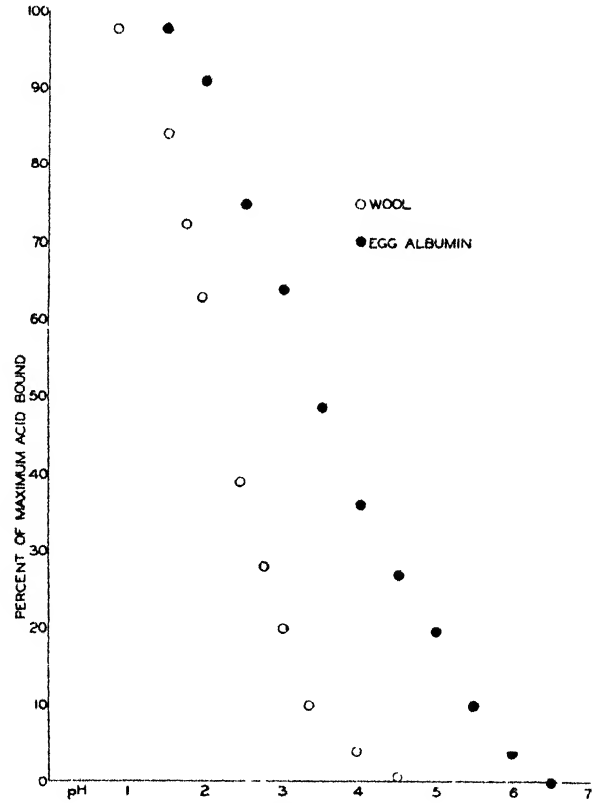


Figure 18

to identify each portion of the curves at high ionic strength with one of the several kinds of dissociating groups contributed by the constituent amino acid residue.

The titration curves for wool keratin obtained by Steinhardt and Harris are shown in Figure 16. Curve A represents the acid-base-binding capacity of purified wool made in the absence of salt, and Curve B the values taken in its presence. With relation to these curves, Steinhardt and Harris point out

that the maximum acid-binding power, independent of the ionic strength, is 0.82 milliequivalent per gram, and that the maximum base-binding capacity is greater than 0.78 millimol. Curve A shows that no appreciable acid or base is bound in the pH interval 5.0 to 10.0, but the amount bound increases sharply as these limits are exceeded. Curve B (values taken in the presence of salt) shows that the point of zero combination is well defined—pH 6.4—and further that the acid or base bound increases gradually with change in pH

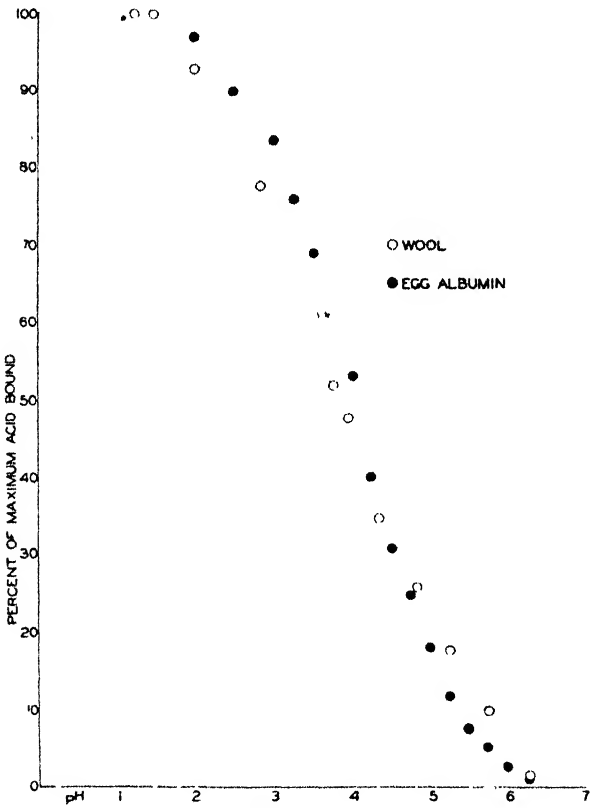


Figure 19

value, the slope of the curves being much less than that of Curve A. If different ionic strengths are used, the position of the titration curves with respect to pH axis changes and these differences are greater than can be attributed alone to the effect of salts on the dissociation of the acids. Steinhardt and Harris further state: "This approach to stoichiometric dependence of the acid bound on the concentration of anions as well as of hydrogen ions accounts for the greater steepness of the titration curve when the source of both ions is the acid alone."

These workers also explain this phenomenon on the basis of the Donnan equilibrium. From such analysis, they concluded that the position of the titration curves with respect to the pH axis, as the ionic strength increases, should approach as a limit the position of the titration curve of a so-called soluble protein-albumin, globulin or gelatin. In other words, at high ionic strength the titration curves of such fibrous proteins as keratin or collagen should correspond to the titration curve of, say, gelatin and should show a gradual change in acid or base binding with change in pH value, and a sharply

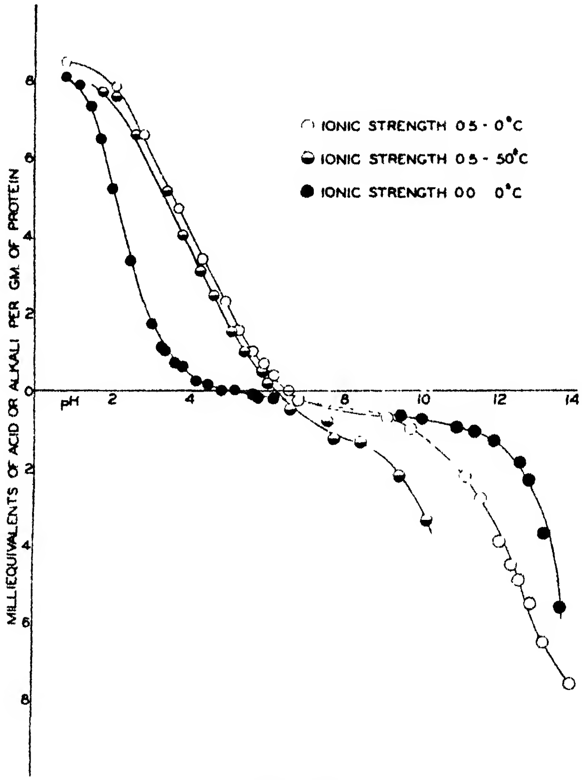


Figure 20

defined nil or isoelectric point. The gelatin titration curve shown in Figure 24 illustrates this similarity. Steinhardt and Harris show this comparison in a very clear manner and Figures 18 and 19 indicate the phenomenon for egg albumin and wool—both in the absence and presence of salt. These curves are self-explanatory and show clearly beyond doubt that if a high salt concentration is employed, the acid or base bound by wool corresponds closely in trend to that of the albumin.

The detailed analysis of the data taken by Steinhardt and Harris leads to the final conclusion that when acid or base binds with wool proteins, such combination occurs with the free carboxyl, imidazole, amino and guanidino groups of the side chains, but that for wool proteins no combination of base takes place with the phenolic groups of the tyrosine present in the pH range studied.

In a further investigation of the acid- and base-binding power of wool proteins, Steinhardt, Fugitt and Harris[69] studied the effect of temperature upon such combination. They found the data supported their earlier assumptions, namely that the free carboxyl and amino groups are completely ionized when in the combined state or, in other words, at the isoelectric point the wool exists in the zwitterion state. These investigators found that temperature plays but little part in the acid-binding zone, but an important one in the base fixation range. Figure 20 illustrates this phenomenon. From data obtained, they calculated the heat of dissociation, using the formula

$$H\frac{T_2}{T_1} = 4.5787\frac{T_2+T_1}{T_2-T_1}\log K.$$

By making the reasonable assumption that the titration curves of proteins are defined by the relative acid strength of distinct, non-overlapping sets of similar dissociating groups, values of $\Delta \log K$ in each of two main regions of pH values were obtained. Thus Steinhardt, Fugitt and Harris calculated the heats of dissociation in wool dissociating at pH values greater than 6.0.

Table 19.

0° to 25° C.			25° to 50° C.		
Millimol base bound per gram	pH	H^{25}	Millimol base bound per gram	pH	H^{25}_{50}
0.1	0.75	11,190	0.1	1.47	25,820
.2	.81	12,070	.2	1.04	18,310
.3	.85	12,640	.3	0.84	14,800
4	.88	13,100	.4	0.78	13,720
.5	.89	13,210	.5	0.84	14,800
.6	.83	12,380			
.7	.81	12,030			
Average		12,380			

These data show the calculated heats of dissociation of the two groups; the carboxyl group being some 2500 calories and the amino group being some 14,000 calories.

In 1941, the same investigators[69] studied carefully the relative affinities of the anions of strong acids for wool protein. Their data for hydrochloric, trichloracetic, picric and flavianic acids are shown in Figure 21. Steinhardt, Fugitt and Harris found that definite differences exist between the positions of the wool titration curves obtained with various strong acids with respect

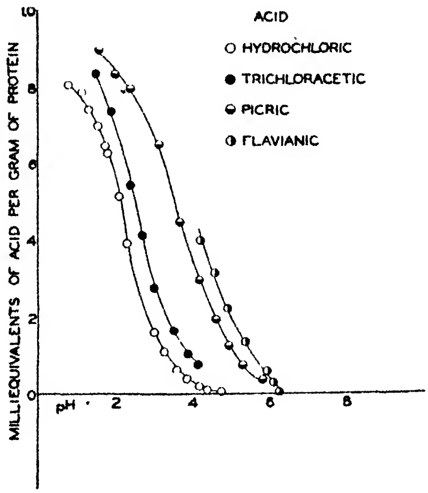


Figure 21

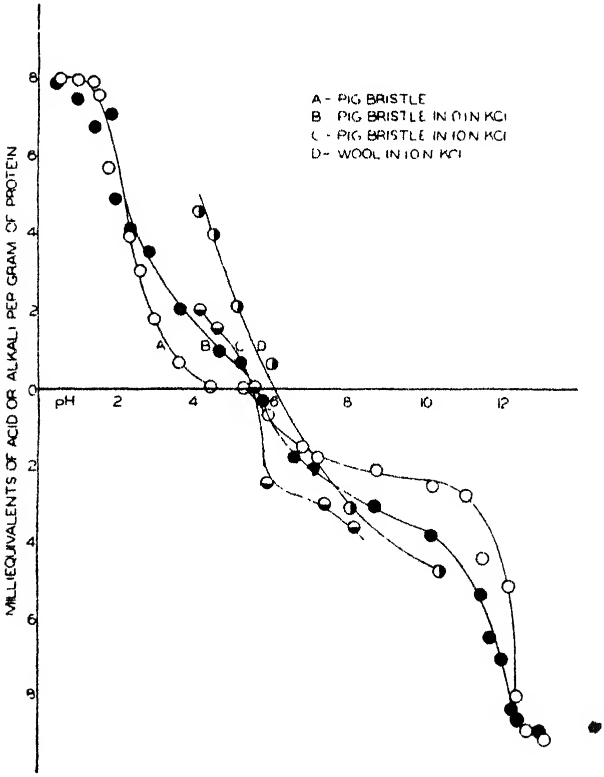


Figure 22

to the pH axis and that such wide difference could be ascribed to definite variations in the anion dissociation constants which characterize the corresponding protein-salt combinations. These investigators suggest: "The affinity of anions for proteins appears to increase with the dimensions of the anion, and is higher in aromatic than in aliphatic ions of the same size."

Theis and Jacoby in 1941[70] and 1942[71] made an extensive investigation of the acid- and base-binding capacity of certain keratin-containing proteins. The early study was confined to Chinese pig bristles. These bristles were thoroughly degreased with acetone and alcohol before experimental treatment. The technique used for this study was identical with that described previously for silk fibroin. The data taken are given in Table 20 and Figure 22.

Table 20. H^+ or OH^- Bound by Bristles.

Without Salt		With Salt (0.1 N KCl)	
pH	Ml of 0.1 N Acid or Alkali bound per Gm of Protein	pH	Ml of 0.1 N Acid or Alkali bound per Gm of Protein
0.55	8.00	0.45	7.90
1.00	8.00	1.00	7.50
1.40	7.95	1.40	6.70
1.50	7.60	1.85	7.10
1.80	5.70	1.95	4.90
2.30	3.95	2.35	4.10
2.55	3.00	2.80	3.50
2.95	1.75	3.60	2.05
3.60	0.65	4.60	+0.95
4.40	0.00	5.20	+0.65
5.25	0.00	5.75	−0.30
5.50	0.00	6.60	−1.80
5.90	0.70	7.10	2.10
6.80	1.55	8.70	3.05
7.20	1.80	10.20	3.80
8.75	2.15	11.45	5.30
10.20	2.50	11.70	6.40
11.10	2.75	12.00	6.95
11.55	4.35	12.25	8.25
12.20	5.10	12.40	8.50
12.40	7.90	13.00	8.80
12.60	8.80		
13.10	9.00		

	pH value of Curve A	B	C
0.1 millimol acid fixed	3.3	4.5	4.7
0.2 millimol acid fixed	2.8	3.8	4.2

Curve A shows the complete titration curve for the system in the absence of salt. This curve shows a positive maximum acid binding at pH 1.0, an isoelectric zone in the pH range 4.4 to 5.6, alkali binding at pH values greater than 5.6, and finally a maximum base binding at pH 13.0. The value obtained for maximum acid binding of 0.800 milliequivalent per gram is only slightly lower than the theoretical calculation 0.856 obtained by Steinhardt and Harris for wool. It is of course possible that the particular hair (pig bristles) used in these studies contained less arginine, lysine or histidine. Jordan-

Lloyd and Bidder showed a value of 0.65 milliequivalent acid bound per gram of horse hair. Steinhardt and Harris showed a maximum acid binding of 0.80 milliequivalent per gram of purified wool. The data of Theis and Jacoby show that in the pH range 5.6 to 11.0, 0.275 milliequivalent of base are bound —a value approximately approaching the theoretical dicarboxylic acid data. At pH values greater than 11.0, however, a greatly increased base binding obtains, indicative of a second type of reaction. Since most hair normally contains tyrosine, the phenolic group of tyrosine may possibly react with base at pH values greater than 11.0, as suggested by Steinhardt and Harris. In the pH range 11.0 to 13.0 some 0.90 milliequivalent of base are bound. This maximum base-binding value is a very positive one.

Table 21. Acid- or Base-binding Capacity of Wool.

Equilibrium pH	Ml 0.1N Acid per gm. Protein	Equilibrium pH	Ml 0.1N Acid per gm. Protein
0.8	+8.3	6.3	− .4
1.4	+8.2	6.7	− .6
2.0	+6.7	7.1	−1.0
3.1	+4.2	7.4	−1.1
4.2	+2.0	7.7	−1.1
4.8	+ .8	8.8	−1.2
5.2	+ .4	9.5	−1.2
5.6	+ .2	10.5	−2.2
5.8	+ .1	11.2	−3.4
6.1	− .2	11.8	−3.6
		12.4	−4.7

Curves B and C of Figure 22 illustrate the effect of ionic strength, namely $0.1N$ and $1.0N$ KCl. As can be seen, Theis and Jacoby found that the presence of salt does not affect the maximum acid or base binding, the salt affecting only the manner in which the maximum value is approached. This fact is shown in Curve B. Curve C ($1.0N$ KCl) gives data only in the so-called pH stability zone and shows the drastic effect of salt in this zone.

If a comparison is made of the pH values at which 0.1 and 0.2 millimol of acid are fixed for the three curves shown in Figure 22, an interesting shift is noted.

The addition of salt also caused a shift in the curves in the alkaline zone and this shift is particularly noticeable at pH values at which 0.2 millimol of base are bound. As noted before, it is necessary to have sufficient anions present to bring about real acid-base binding in the so-called isoelectric zone.

In a further study of the hair keratins, Theis and Jacoby[72] investigated the acid- and base-binding of purified wool. The wool for this investigation was thoroughly degreased with acetone and alcohol and was then washed with running water to free it of all occluded material; it was then pressed and air-dried. The technique employed for the investigation was similar to that used for the study of the Chinese pig bristles. In this work, all acid or base

solutions were made $0.1N$ with respect to potassium chloride. Table 21 and Figure 23 show the data obtained.

These data show a positive maximum acid fixation value of 0.83 millimol of acid bound per gram of protein at pH values less than 1.5. This value is well in line with the calculated theoretical value of 0.856 used by Steinhardt and Harris. The titration curve shows first, a sharp isoelectric (isoionic) point at pH 5.9; secondly, a rather broad plateau zone in the pH range 7.0 to

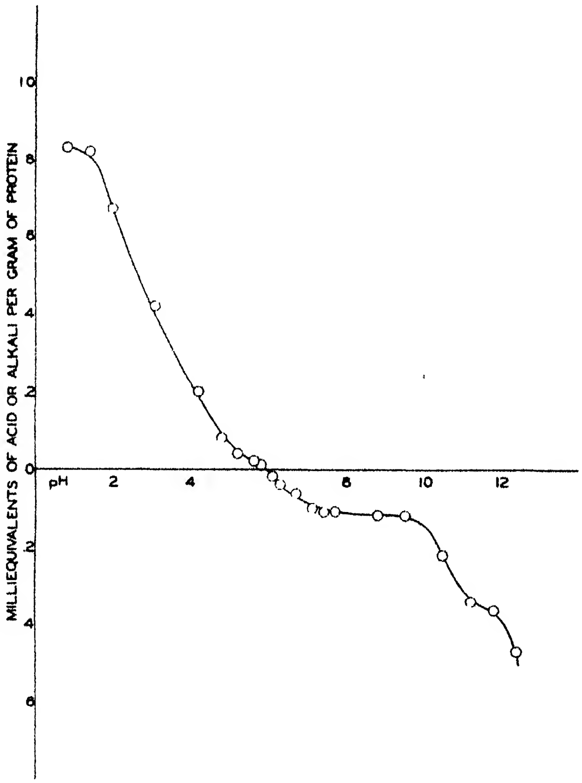


Figure 23

10.0, a base-binding value of approximately 0.13 milliequivalent per gram; and thirdly, a sharp increase in base fixation at pH values greater than 11.0. Above pH 12.5 the hair is hydrolyzed and for that reason titration values beyond this point were not obtained. The titration curve shows in a very positive manner that at pH values less than 5.9 acid is bound whereas above 5.9 base is bound, and that no isoelectric zone is present, as has been suggested by Jordan-Lloyd and Bidder. With the exception of a slightly more acid

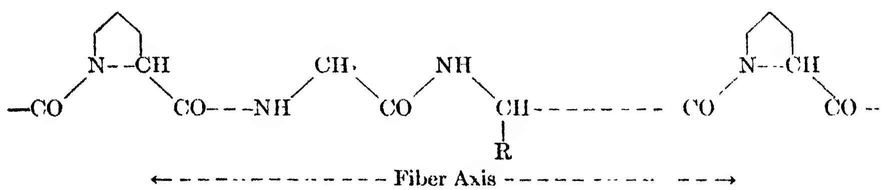
isoionic point, the titration curve for wool obtained by Theis and Jacoby corresponds closely to that of Steinhardt and Harris.

The Acid- and Base-binding Capacity of Collagen

X-ray analysts have investigated the diffraction patterns of collagen and gelatin and have found that this pattern is somewhat different from that of keratin. Astbury in 1932 concluded that every third amino-acid residue in the collagen structure was glycine, and that every ninth might be a proline or oxyproline residue. Astbury's[3] analysis indicated a residue length of about 2.9 Å versus 3.5 Å for the fully extended polypeptide structure. Astbury maintained that it was the preponderance of imino residues that constrict the collagen backbone. He illustrates the collagen structure as follows:

-------P—G—R—P—G—R—P—G—R-------

in which P stands for either proline or hydroxyproline, G for glycine and R for one or other of the remaining residues. This sequence may be written structurally:



The collagen structure is not nearly as compact as that found for silk fibroin or hair keratin and must be considered as just slightly coiled. The polypeptide chains are oriented parallel to the fiber axis and are held together by means of hydrogen bonds (van der Waals forces) and salt linkages between certain charged centers along the backbone. The origin of the charged centers must be the content of arginine, lysine, histidine, glutamic and aspartic acids. The acid- or base-binding power of collagen has to do primarily with these particular acidic or basic amino acids.

During the past 15 years, a number of investigators have analytically studied the amino acid content of gelatin and collagen. Much of the data

Table 22. Amino acid Frequencies in Gelatin.

Amino acid	Wt. (%)	Mol Wt.	Gm. Mol.	Frequency
Glycine	25.5	75	0.34	3 $(2^0 \cdot 3_1)$
Proline	19.7	115	0.17	6 $(2^1 \cdot 3_1)$
Hydroxyproline	14.4	131	0.11	9 $(2^0 \cdot 3_2)$
Alanine	8.7	89	0.098	9 $(2^0 \cdot 3_2)$
Arginine	9.1	174	0.052	18 $(2^1 \cdot 3_2)$
Leucine-isoleucine	7.1	131	0.054	18 $(2^1 \cdot 3_2)$
Lysine	5.9	146	0.040	24 $(2^3 \cdot 3_1)$

used today dates back to the early results of Dakin.[25] Bergmann[11] in a very systematic investigation has thrown new light upon the arrangement and periodicity of the amino acid residues contained in the polypeptide chain of gelatin. Table 22 shows the frequency of occurrence of certain amino acids contained in gelatin.

Atkin[6] in 1933 discussed the composition of gelatin and collagen and concluded that its molecular weight should be approximately 34,500. Braybrooks[15] in 1939 recalculated Atkin's data and showed that the collagen molecule should contain 2 histidine, 12 lysine, 16 arginine, and 49 dicarboxylic acid residues. He also calculated the theoretical titration curve of collagen. Highberger[39] in 1937 determined the basic amino acid content of purified collagen and obtained results somewhat at variance with those of other investigators, which might have been due to a difference in material studied. Table 23 shows the amino acid content of gelatin and collagen as obtained by several analysts, expressed as percentage of dry material.

Table 23. Amino Acid Analysis of Gelatin and Collagen.

Amino Acid	I Gelatin*	II Gelatin†	III Collagen‡	IV Collagen§
Glycine	25.5	26.5	24.7	—
Alanine	8.7	8 7	—	—
Valine	—	—	—	—
Aspartic acid	3.4	—	3.5	—
Glutamic acid	5.8	—	5.7	—
Arginine	8.2	9.1	8.4	7.69
Lysine	5.0	5.9	4.7	4.12
Histidine	0.9	—	0.6	0.30
Tyrosine	—	—	1.0	—
Proline	9.5	17.5	18.9	—
Oxyproline	14.1	14.4	10.8	—
Leucine	7.1	7.1	—	—
Phenyl alanine	1.4	—	—	—
Serine	0.4	—	—	—
Amid N.	0.5	—	—	—

* Astbury and Atkin, *Nature*, 132, 348 (1933).[3]
† Bergmann and Stein, *J. Biol Chem*, 128, 217 (1939).
‡ Schneider, *Collegium*, 97, 97 (1940)
§ Highberger, Stiasny Festschrift (1937).

Since for acid- and base-combining capacity only the terminal charged groups of the acidic and basic amino acids are important, the authors have taken column I of Table 23 and calculated the theoretical acid- and base-binding power from these values. These are given in Table 24.

Atkin suggested that the acids still unaccounted for must be of relatively high molecular weight. It is still unknown whether these missing acids contain more than one carboxyl group, but Atkin suggests they must at least contain hydroxyl groups. If the amounts of arginine, lysine, and histidine are added together, it is then found that the sum agrees rather well with the maximum amount of acid bound, as determined by a number of investigators.

However, the sum of the amounts of glutamic and aspartic acids as given in Table 24 does not in any way agree with the maximum base fixation as given in the literature. Such difference would lead to the belief that the unknown acids must contain additional base-binding groups. Braybrooks, in discussing Atkin's research, presents the idea that since gelatin has an isoelectric point at pH 4.7, there must be more acidic than basic groups, particularly since the basic groups are "stronger."

Table 24. Amino Acid Content of Gelatin and Calculated Theoretical Acid or Base Fixation Values.

Amino acid	Per cent	Millimol per gm
1. Aspartic acid	3.4	0.257
2. Glutamic acid	5.8	0.394
3. Tyrosine	1.0	0.055
4. Amid nitrogen	0.5	0.358
1+2−4		0.293
1+2+3−4		0.348
5. Arginine	8.2	0.471
6. Lysine	5.0	0.343
7. Histidine	0.9	0.060
5+6+7		0.874
5+6		0.814

In 1931, Hitchcock[42] measured the acid- and base-binding power of gelatin at 30° C. This curve is shown in Figure 24. It is typical of the results obtained for other soluble proteins, such as egg albumin and serum albumin. In the Hitchcock curve for gelatin, no break is evident at the isoelectric point, and this is in agreement with the fact that soluble proteins have sharply defined isoelectric points in contradistinction to the fibrous proteins. The trend of this curve indicates a maximum acid binding of approximately 0.96 milliequivalent per gram of protein; an isoelectric point at pH 4.7; a rather broad plateau in pH zone 6.5 to 8.5; and an approximate base fixation of 0.95 milliequivalent of base per gram of protein at pH 12.5. The plateau region near pH 8.0 appears to mean that one set of ionizable groups has given off all of its available protons in this region.

As has been shown for the dissociation curve of an amino acid or a peptide, each section indicates a single group capable of adding or donating a proton. In the case of a protein, such a simple relation is not possible, because the constants are so close to each other. Thus in Figure 24, on the alkaline side of the isoelectric point protons are given off in steps, corresponding to 0.36 milliequivalent at approximately pH 8.0 and an additional 0.54 milliequivalent at pH 12.0. Most investigators agree that the very central zones of the protein dissociation curve represent a truly reversible proton donor and acceptor condition, but some doubt exists as to whether the protein retains its original qualities at low and high pH values.[68]

Jordan-Lloyd and Bidder investigated the dissociation curve of collagen.

These data are shown in Figure 15. For collagen they show only an indication of a maximum acid fixation of better than 0.8 milliequivalent per gram, an isoelectric zone in the pH range 4.5 to 6.5, a broad plateau in the pH zone 8.0 to 11.0, and no real base-finding maximum even at pH 13.0.

The data obtained by Highberger,[38] using a special, highly purified collagen, is quite different from similar data obtained by any other worker in this field. His curve for the purified collagen shows no real indication of a maximum

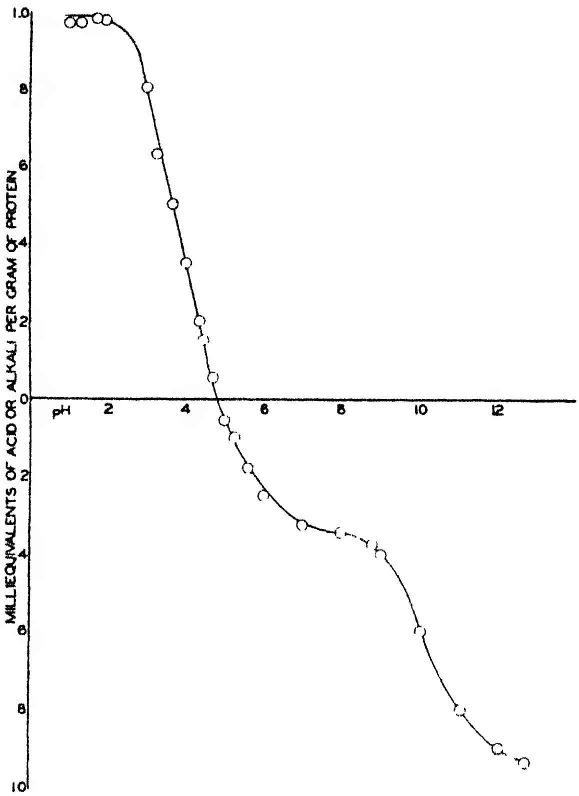


Figure 24

acid fixation, but an actual decrease in the value at pH values less than 2.0; an isoelectric zone in the pH range 4.9 to 6.0; a broad plateau in the range pH 7.0 to 10.1; and no real values for maximum base binding in the more alkaline zone. Highberger questions the validity of applying the potentiometric method, used for the soluble proteins, to the heterogeneous fibrous protein systems. Highberger's point is well taken, since the low values of acid fixation at pH values less than 2.0 might be ascribed to variations in the

ratio of acid and water absorbed by the protein. A similar condition obtains at pH values greater than 12.5.

Atkin[7] in 1937 studied the acid- and base-binding capacity of deaminized collagen. In reference to the titration curve obtained, Atkin refers to the portion of the curve between the isoelectric point and pH 8.0 as representing free carboxyl groups in excess of those required to form salt linkages with basic groups in adjacent chains. He drew certain conclusions from his titration curve, which the authors believe apply generally to all collagen titration curves; for this reason they are listed:

(a) From the isoelectric point to pH 2.0 back titration of charged carboxyl groups occurs.
(b) From the isoelectric point to pH 7.5 straight titration of free and unionized carboxyl groups takes place.
(c) From pH 7.0 to 9.0, back titration of histidine occurs.
(d) From pH 9.0 to 12.0, liberation of protons from the charged amino groups of lysine occurs.
(e) At pH values greater than 12 0, back titration of the basic ions in arginine occurs.

In 1940, McLaughlin and Adams[56] investigated the sulfuric acid-binding capacity of collagen. For this study, these investigators used a unique method of determining the bound or fixed acid. After equilibrium has been attained, the collagen-acid compound was pressed twice at 5000 pounds per square inch in a hydraulic press. The pressed material was then air-dried, ground to a fine powder and then analyzed for hide substance and bound sulfate. Although modifications of this method were used by Theis and Jacoby[71] for investigations of silk fibroin, hair keratin and collagen, the original method as used by McLaughlin and Adams is here given verbatim.

"Whole, bated calf skins were brought to pH 5.0 by the addition of small increments of hydrochloric acid. They were then washed until free of uncombined neutral salts, when they showed a pH value of 6.5. They were then cut into strips and placed in frequently changed portions of acetone until thoroughly dehydrated. The acetone was allowed to evaporate from the skin at room temperature, and the now dry strips were cut into pieces one-half inch square, which were then well mixed.

"Ten grams calf skin hide substance in the form of squares prepared as described above were weighed into an 8-ounce stoppered bottle. Distilled water was then added and the hide substance was allowed to soak 30 minutes. The required amount of $N/1$ H_2SO_4 was then added and a final volume of solution of 100 ml was measured. The bottles were then continuously agitated for 24 hours in a water bath at 19 rpm and 70° F, this time of agitation having been found sufficient for attainment of equilibrium. At the end of 24 hours,

the samples were removed and poured into funnels and allowed to drain. The volume of the drained solution was noted and its pH value electrometrically determined. The drained squares were then pressed twice in a hydraulic press at 5000 lbs pressure. They were air dried after pressing and were ground in a Wiley mill. The acid SO_4 and nitrogen were then determined on each pressed sample, and the acid content on the hide substance basis calculated therefrom. The detail of the acid SO_4 determination is as follows: a 1.0-gram sample is weighed into a 125-ml Erlenmeyer flask and treated with 10 ml HNO_3. The sample is then boiled on a hot plate until the volume is about 5 ml; it is then cooled, treated with 10 ml conc. HCl and again evaporated

Table 25.

Grams H_2SO_4 given per 100 gms Collagen	Grams Acid Fixed	Grams Acid Unfixed	pH of Residual Acid Solution
4.00	3.72	0.28	2.35
5.00	4.44	0.56	2.05
6.00	4.72	1.28	1.75
6.40	5.14	1.26	1.75
7.20	5.24	1.96	1.60
8.00	5.38	2.62	1.50
8.80	5.82	2.98	1.40
9.60	5.92	3.68	1.30
10.40	5.93	4.47	1.25
11.20	6.06	5.14	1.20
12.00	6.08	5.92	1.15
13.00	6.37	6.63	1.10
14.00	6.44	7.56	1.08
15.00	6.46	8.54	1.00
16.00	6.65	9.35	0.98
17.00	6.66	10.34	0.92
18.00	6.83	11.17	0.90
19.00	7.00	12.00	0.85
20.00	8.78	11.22	0.90
21.00	8.88	12.12	0.83
22.00	9.50	12.50	0.80
23.00	9.72	13.28	0.79
25.00	9.50	15.50	0.73

to a volume of 5 ml. It is then transferred to a 600-ml breaker and neutralized with NH_4OH (litmus). After diluting to about 300 ml and adding 5 ml conc. HCl, the sample is boiled and treated with 10 ml 10 per cent $BaCl_2$. After standing overnight the sample is filtered through a Gooch crucible, washed five times with hot water and then ignited for one hour. Nitrogen was determined by the usual Kjeldahl/Gunning method. The original prepared skin squares showed a negligible sulfate content."

The data obtained in the investigation of McLaughlin and Adams are given in Table 25 and Figure 25.

Regarding the data shown above, McLaughlin and Adams summarize it as follows:

"When collagen is treated with sulfuric acid in amounts ranging from 4

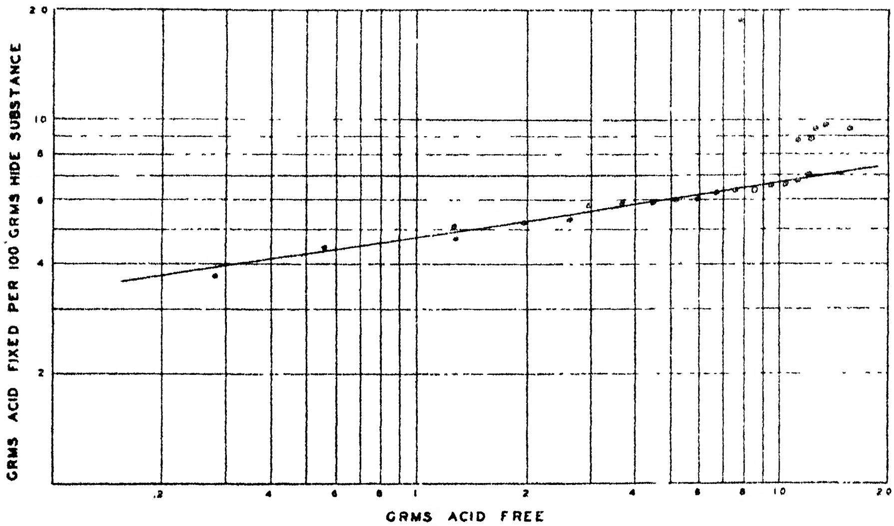


Figure 25

Table 26.

% Acid SO_4 given	% Na_2SO_4 given	% Acid SO_4 Fixed	pH Exhaust Solution	% which Acid SO_4 is of total SO_4 in original solution
10.00	0.00	5.86	1.32	100.0
10.00	5 00	5.72	1.35	74.7
10.00	10.00	5.51	1.41	59.7
10.00	20.00	5.02	1.52	42.5
10.00	30.00	5.15	1 60	33.0
10.00	40.00	5 01	1.63	27.0
10.00	50.00	5.01	1.68	22.9
10.00	60.00	4.98	1.68	19.8
10.00	80.00	4.87	1.70	15.6
10.00	100.00	4 84	1.78	12.9
15.00	0.00	6 97	1.00	100.0
15.00	5.00	6.78	1.01	81.6
15.00	10.00	6.51	1 08	68.9
15.00	20.00	6.37	1.11	52.6
15.00	30.00	6.08	1.20	42.5
15.00	40.00	6.02	1.30	35.7
15.00	50.00	5.67	1.33	30.7
15.00	60 00	5.48	1.35	27.0
15.00	80.00	5.37	1.40	21.7
15.00	100.00	5.31	1.40	18.1
20.00	0.00	7.63	0.90	100.0
20.00	5.00	7.56	0.90	85.6
20.00	10.00	7.71	0.99	74.7
20 00	20.00	7.16	0.99	59.7
20.00	30.00	6.85	1.13	49.6
20.00	40.00	6.35	1.19	42.5
20.00	50.00	6.33	1.21	37.2
20.00	60.00	6.15	1.25	33.0
20.00	80.00	5.97	1.30	27.0
20.00	100.00	5.88	1.35	22.8

to 19 per cent, the log of acid bound plotted against the log of the unbound yields a straight line. When 19 per cent sulfuric acid is given collagen, 7 per cent is fixed, under the experimental conditions outlined herein. When amounts of sulfuric acid greater than 19 per cent are given, the amount of acid fixed increases and may reach around 9.50 per cent.

The reactivity of collagen and acid is a subject of great importance in both the theory and practice of tanning."

In 1942, McLaughlin and Adams,[57] in a subsequent study, investigated the effect of sodium sulfate upon sulfuric-acid fixation of collagen. They found that the presence of salt decreased the amount of H_2SO_4 bound by the collagen from a given acid solution. Table 26 and Figure 26 give their data, where percentages given are based on collagen.

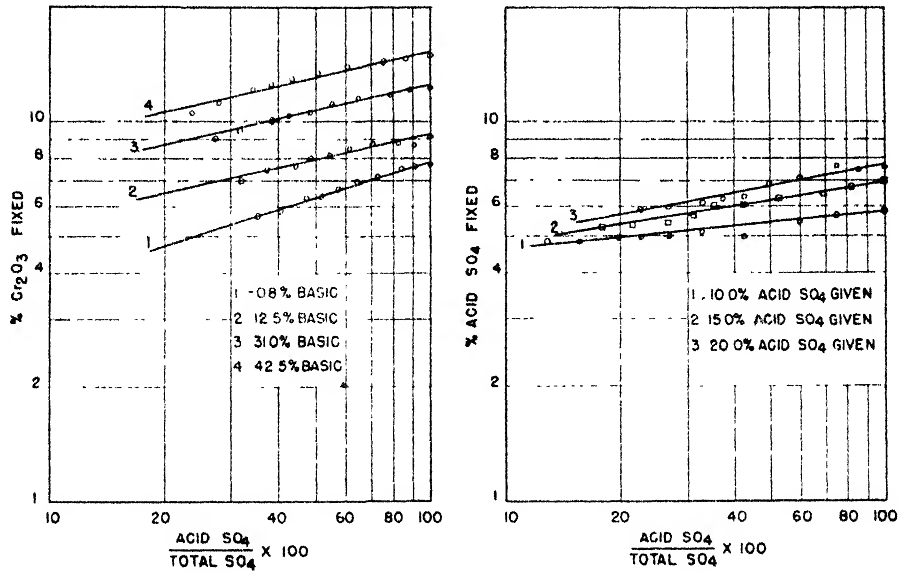


Figure 26

From a cursory examination of these data, it might appear that these investigators are at variance with data presented by other workers in this field. Such is not the case, however, since if these data are plotted versus equilibrium pH value, it is found that the neutral salt actually increases acid fixation in a given pH range.

In 1941, Theis and Jacoby[71] made an investigation of the acid- and base-binding capacity of animal skin collagen. For this investigation, these workers used specially prepared skin collagen. This collagen was prepared by properly soaking goat skin and then liming it in a sharp lime for 24-48 hours. The hair and adhering flesh was removed—the skin then washed

Table 27. Acid Bound by Collagen.

0 0% Salt 48-hr equilibrium period		0 1N KCl 48-hr equilibrium period		0 1N KCl 8-hr equilibrium period	
Equilibrium pH	ml 0.1N acid per gram protein	Equilibrium pH	ml 0.1N acid per gram protein	Equilibrium pH	ml 0.1N acid per gram protein
0.95	9.7	0.75	9.6	0.80	8.8
1.50	8.6	1.25	8.8	1.05	8.8
2.20	7.6	1.85	8.7	1.45	8.8
2.90	5.9	2.60	8.2	2.55	8.7
3.40	3.5	3.15	7.5		
3.50	3.2	4.10	5.1		
3.90	1.3	4.70	3.8		
4.20	0.30	5.80	0.75		
5.30	0.0	6.30	0.60		
5.60	0.0	7.10	0.20		
5.80	0.0	7.60	0.0		
6.70	0.0	8.80	−0.65		
7.05	0.0	9.90	−2.1		
7.35	0.0	10.55	−2.6		
7.50	−0.15	11.45	−2.6		
7.70	−0.40	11.75	−2.8		
8.80	−0.50	12.30	−4.4	12.50	−3.5
10.00	−0.60	12.60	−4.7		
10.40	−0.90	12.75	−4.7		
11.00	−1.1				
11.60	−1.6				
11.90	−2.0				
12.20	−2.7				
12.40	−4.3				
12.70	−4.6				

thoroughly in running water until free of soluble $Ca(OH)_2$. The thoroughly washed skin was then treated with acetic acid so as to remove bound $Ca(OH)_2$. After again washing, the skin was dehydrated by frequent changes of acetone and alcohol. The dehydrated material was then cut into pieces 0.5 × 1.5 inches for use.

The experimental technique used by Theis and Jacoby was identical with that described earlier for their investigation of the acid or base binding of

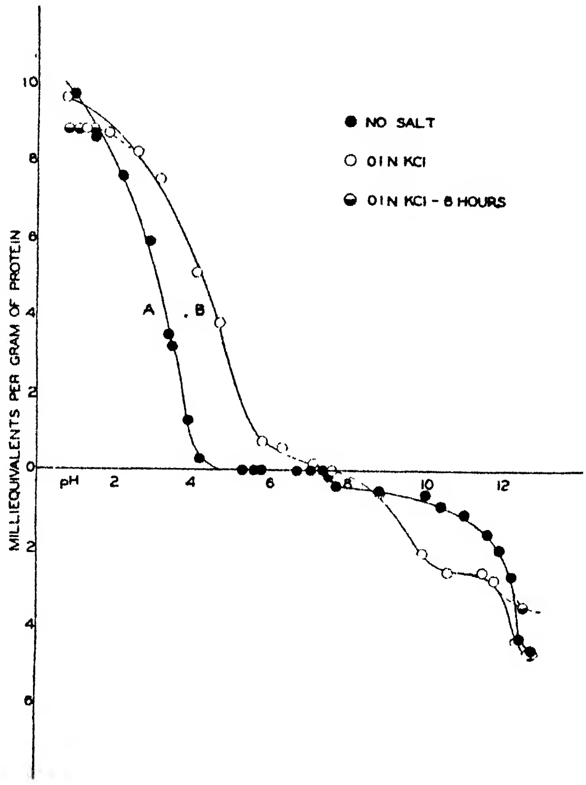


Figure 27

silk fibroin and hair keratin. In most cases an equilibrium period of some 72 hours' contact with the acid or alkali was used. However, at pH values less than 2.0 and greater than 12.0, an 18-hour period sufficed. This decreased period of contact was necessary to retard hydrolysis and simplification of the protein at the low and high pH values.

Table 27 and Figure 27 give the data obtained by Theis and Jacoby for the collagen investigated by them. Curve A of this figure represents aqueous acid or base solution, and Curve B represents that of acid or base solution

maintained $0.1N$ with respect to potassium chloride. Both curves indicate a maximum acid fixation of approximately 0.94 milliequivalent per gram of protein at pH values less than 1.0 and a maximum alkali fixation of approximately 0.46 milliequivalent per gram of protein at pH values greater than 12.8. The curve for the aqueous solutions (Curve A) shows a sharp slope in the pH range 2.0 to 4.2, a broad isoelectric zone in the pH range 4.6 to 7.5, a rather broad plateau in the alkali fixation range pH 8.0 to 11.0, and a sharp increase in alkali fixation at pH values 11.0 to 12.0. Curve B, representing salt solutions of acid or base, while showing the same maximum acid or base fixation, has an entirely different trend in other sections of the curve. It shows a much decreased slope in the pH range 2.0 to 6.0, a definite isoelectric point at pH 7.6 and a much increased alkali binding in the pH range 8.0 to 10.0, with a rather flat region between pH 10.0 and 12.0.

The analysis of the collagen for arginine, lysine, histidine, aspartic acid, glutamic acid and tyrosine indicates that there should be a maximum acid fixation of approximately 0.874 milliequivalent per gram of protein and a maximum base fixation of 0.348 milliequivalent per gram of protein. The values given in Figure 27 show approximately an 11 per cent greater value for acid fixation and some 30 per cent greater values for base fixation. The values for maximum acid fixation, however, are approximately those obtained by Hitchcock for gelatin. However, with regard to base fixation, the values shown in Figure 27 are decidedly more in line with analytical data than those given by Hitchcock, by Jordan-Lloyd and Bidder, and by Highberger.

The displacement of the titration curves using potassium chloride solution rather than a mere aqueous solution is undoubtedly caused by the salt effecting a more uniform diffusion of the acid or base into the collagen and can be readily explained by the Donnan membrane equilibrium theory. By using potassium chloride solutions of acid or base, a definite isoelectric point is attained. Beek,[10] Highberger[40] and Theis[73] have shown by electrophoretic studies that the true isoelectric point of collagen is at pH 7.6 to 7.8. However, standard titration curves for collagen have always indicated an isoelectric point approximating pH 5.0, and it has been difficult to correlate values obtained by this means with those obtained by electrophoresis. Curve B shows rather clearly that the isoelectric point obtained by Theis and Jacoby agrees well with values obtained by electrophoretic studies.

Further investigation by Theis and Jacoby showed that after some 20 hours contact with acid at pH 0.85 or pH 12.5, collagen was attacked and hydrolysis and simplification occurred. Data obtained showed a sharp increase in acid fixation taking place after a 20-hour contact period. For this reason, Theis and Jacoby in later studies used a 20-hour reaction period in the more acid and in the more alkaline regions in place of the usual 72-hour period for the less acid or alkaline zone. The dotted portion of the curves

shown in Figure 27 indicates the most probable values of maximum acid- or base-binding capacity of the collagen. These corrected maximum values for acid- or base-binding power are, then, 0.85 milliequivalent of acid and 0.35 milliequivalent of base bound per gram of protein, and are well in line with the analytical data shown in Table 27.

The Acid and Base Fixation of Denatured Collagen

Theis[74] and his students have shown that when collagen in the presence of moisture is heated to 65° C or above, irreversible shrinkage takes place. Under such conditions, it may be said that the collagen has been heat-denatured. Gottschlich[29] and Jensen[44] call this phenomenon "thermal rigor,"

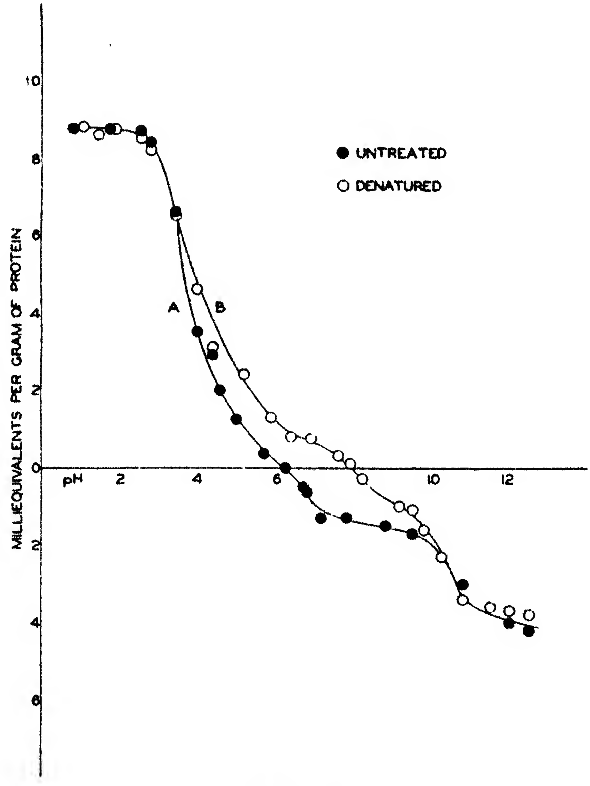


Figure 28

and Gustavson[33] uses the term "hydrothermal stability." Theis found that shrunken collagen fixed less chromium salt than did untreated collagen. For this reason, Theis and Jacoby[75] turned their attention to an investigation of the acid- and base-binding capacity of heat-shrunken protein. For this study

they used a special collagen, the preparation of which has been described earlier in this chapter. The collagen was shrunk before its equilibrium contact with acid or base. Figure 28, covering the data taken, shows:

(1) The particular natural collagen has an isoelectric point at pH 6.3 while the heat-denatured protein has one at approximately pH 8.0. This shift of the isoelectric point to the more alkaline region substantiates Paulings'[60] suggestion, namely, that the isoelectric point, upon denaturing, shifts toward neutrality. Such data might also indicate that upon denaturing the basic groups of the collagen become "stronger" and play a more predominant role. This shift of the isoelectric point is very definite in nature, actually causing a displacement of the titration curve to the more alkaline region of some 1.8 units of pH at this point.

(2) Curve A is the usual characteristic titration curve for native collagen, showing some 0.87 milliequivalent of acid fixed per gram of protein, a plateau in the pH range 7.0 to 9.5, and a maximum base-binding capacity of 0.42 milliequivalent per gram of collagen. Curve B represents the acid- or base-binding of the heat-denatured collagen. This curve shows practically the same maximum acid- and base-binding power as that shown in Curve A. However, the heat-denatured collagen shows a difference in acid and base fixation capacity in the pH region 4.5 to 10.0. In this particular range, the denatured collagen shows increased avidity for the hydrochloric acid and a decreased base-binding power. The data of Theis and Jacoby lend strength to the idea that heat denaturization gives some slight increase in strength to certain of the titratable groups rather than in their number. It appears evident that the strength of the basic groups is materially increased and that that of the acidic groups is decreased.

To explain the phenomenon—namely, the shift of the isoelectric point and the difference in the titration curve from that of native collagen—requires more experimental data than is contained in the literature. Bull[18] points out the confusion existing at the present time regarding this question. Loughlin[54] claims that the titration curve for heat-denatured proteins is identical with that for the native protein, while Chow and Wu[21] claim a real difference exists. Michaelis and Davidson[59] indicate that the isoelectric point of denatured protein is higher than that of native protein. Bull believes that this difference in titration cannot be marked and that the lack of difference argues against any great destruction of the zwitterion structure. Hendrix and Wilson[37] claim a considerable decrease in both the acid- and base-binding capacity of the coagulated protein (egg albumin). As an explanation of the effect of heat denaturization upon the titration curve, Cohn, McMeekin, Edsall and Blanchard[23] suggest that the carboxyl and amino groups may come within each other's sphere of attraction and thus reduce the zwitterion character. Jordan-Lloyd[50] indicates that the value of the isoelectric point

of a protein depends not only upon the number but also upon the proximity and space arrangement of the charged groups; thus denaturization may cause such a change in molecular configuration as to shift the isoelectric point of the protein drastically.

The data of Theis and Jacoby definitely show a change in isoelectric point. Such results are entirely in line with the suggested ideas of Michaelis and Davidson and of Pauling. It may very well be that the dehydration occurring

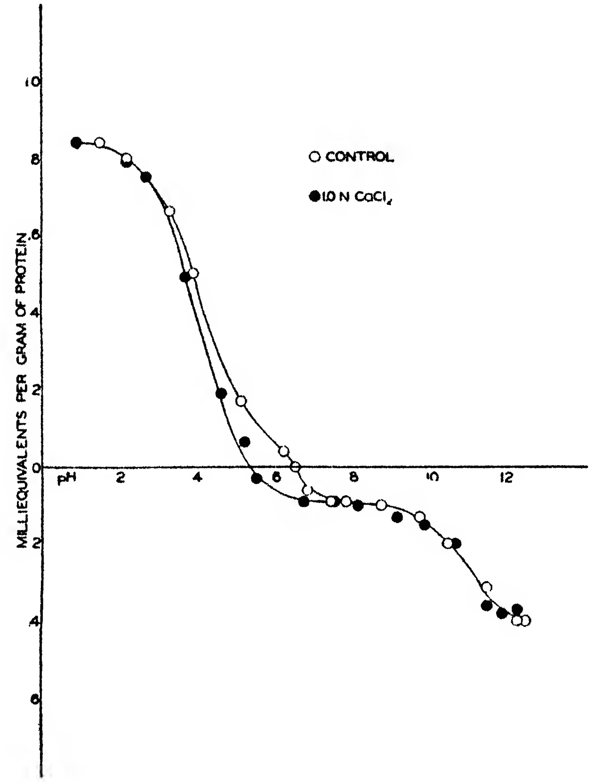


Figure 29

during the supercontraction of the collagen structure during heat denaturization causes a change in the zwitterion linkages, converting a number of them to possible coordinate links. Such coordinate bonds might easily reverse in the higher acid and alkaline regions, thus causing the same acid or base binding at low and high pH values.

Many investigators have found that calcium chloride causes shrinkage not only of native collagen but also of vegetable- and chrome-tanned leathers.

Theis and Jacoby investigated the effect of such salts as potassium chloride, sodium sulfate, and calcium chloride upon the acid and base fixation of collagen. For this work they used acid or base solutions made 1.0N with respect to the salts used. Collagen was placed in such solutions until equilibrium obtained (72 hours), after which the collagen was removed, pressed, dried, and analyzed for H^+ or OH^- bound. Figure 29 shows the data taken, which indicate that the isoionic point of the collagen-$CaCl_2$ compound has been definitely shifted to a more acid point or that the ability of the collagen-$CaCl_2$ compound to bind acid has been lessened while the ability to bind alkali has been increased in the isoelectric zone. The maximum acid or base fixation capacity has not been altered, however, as can readily be seen from the figure. Potassium chloride and sodium sulfate have definitely no effect upon the isoionic point of the collagen compound.

It will be shown later in this chapter that calcium chloride readily combines with collagen in the pH range greater than 3.0 and that potassium chloride combines to a much less degree. Since concentrated solutions of calcium chloride so drastically weaken the structural forces of collagen, it is reasonable to expect certain changes to have occurred which may modify the acid- or base-binding capacity of the collagen-$CaCl_2$ compound. The data definitely indicate a denaturization effect.

Sulfuric Acid Binding of Collagen

In 1940, McLaughlin and Adams studied the binding of sulfuric acid and collagen and in 1942[57] the binding of this acid in the presence of sodium sulfate. These data have already been discussed. In 1941, Theis and Jacoby[76] investigated the collagen-sulfuric acid reaction with the idea of comparing it with collagen-hydrochloric reactivity. Theis and Jacoby found that the titration curve obtained with sulfuric acid was somewhat different from that obtained with hydrochloric acid. Both McLaughlin and Adams and Theis and Jacoby showed that at low pH values more sulfuric acid was bound than hydrochloric acid under the same conditions. Such findings are difficult to explain, since the results of Loeb[63] show the direct opposite. Loeb used his data to explain the correctness of the chemical viewpoint in relation to the protein-acid or -alkali systems. However, Loeb carried his pH values to 2.2-2.4 only, and it is at lower pH values that sulfuric acid shows greatest variations from hydrochloric acid. Figure 30 gives the data taken by Theis and Jacoby for sulfuric acid, sodium hydrogen sulfate and sulfamic acid. This shows that both sulfuric acid and sodium hydrogen sulfate cause a greater maximum H^+ ion fixation than does hydrochloric acid (see Figure 27). Sulfamic acid, on the other hand, gives approximately the same maximum H^+ ion fixation as hydrochloric acid. Steinhardt, Fugitt and Harris,[69] in a study dealing with the relative affinities of the anions of strong acids for wool

proteins, ascribe the difference in their titration curves to wide variation in anion dissociation constants characterizing the corresponding protein-anion combinations. These investigators used such acids as hydrochloric, nitric, trichloracetic, ethyl-sulfuric, etc. They did not make a study of the protein-sulfate combination because of complications caused by the dibasic acid.

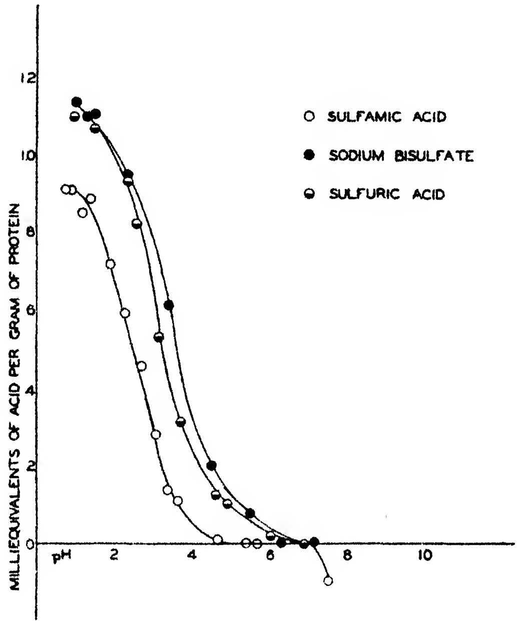


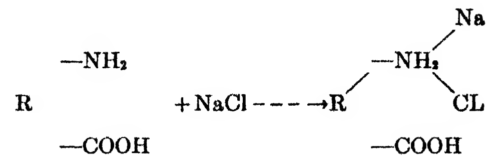
Figure 30

Combination of Salts and Proteins

The actual combination of salts with proteins has been studied by only a few investigators. This is largely because of the difficulties encountered in the actual methods of their determination. Pauli[62] held that neutral salts form adsorption compounds with non-ionized protein molecules in which both ions of the salt were simultaneously absorbed. Loeb[53] postulated, "When neutral salt is added to a gelatin solution on either side of its isoelectric point only a depressing action of that ion which has the opposite sign of the charge to the protein ion is observed," and "At the isoelectric point, *i.e.*, at pH 4.7, gelatin can combine with neither ion of a neutral salt; at a pH $>$ 4.7, only the metal ion of the neutral salt can combine with the gelatin, forming metal gelatinate; at a pH $<$ 4.7 only the anion of the neutral salt is capable of combining with the protein, forming gelatin-acid salts."

Hardy[84] in 1905 postulated the formation of compounds between protein

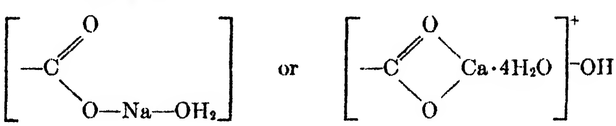
and neutral salts, the combination being between the salt and the amino groups—



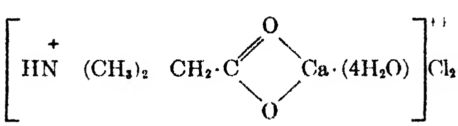
Adolf[1] in 1923 stated that in 0.1*N* solution, four moles of alkali chloride bind one mol of globulin. Pauli[62] and later Leuthardt[52] suggested salt fixation through coordination of the salt with the charged amino group:

$$^{-}OOC\cdot R\cdot NH_3^{+} + Me^{+}X^{-} \dashrightarrow {}^{-}OOC\cdot R\cdot NH_2\cdot MeX + H^{+}$$

It has also been suggested that neutral salts may coordinate at the carboxyl group of the protein. Anslow and King[2] concluded from their studies that the central metallic atom coordinates with either one or both of the oxygen atoms of the carboxyl groups of the amino acid:



Pfeiffer indicates similar coordination for betaine and the haloid salts:



Northrop and Kunitz[61] in 1924-28 studied the combination of a great many salts with gelatin. They found that the activity of an ion in a protein solution could be determined by setting up a Donnan equilibrium and then measuring the total ion concentration outside and inside the membrane. They found, however, that under the best conditions, the error in the final calculated value might be 10 or 20 per cent. Their results, using $CuCl_2$, indicated a maximum fixation of Cu^{++} ion of approximately 0.9 millimol per gram of gelatin, well in line with the equivalent combining power of gelatin for H^+ ion. Identical conclusions were reached for Ca^{++} ion fixation at pH 4.7, but no Ca^{++} ion was bound at pH values less than 3.0. Northrop and Kunitz found no combination of K, Li, or Na.

Greenberg and Schmidt[32] have shown that when casein is dissolved in solutions of the alkaline-earth metals, the transport number of the casein ion is above normal. These investigators conclude that such data indicate the presence of complex metallic-containing casein ions, since some of the metallic

element is carried in a direction contrary to its normal path. Greenberg and Schmidt schematically express the complex as follows:

$$M_a \text{ protein} = XM^{++} + M_{(a-x)} \text{ protein} - 2x$$

where M stands for the alkaline-earth metal, a the valency, and x the amount of the metal dissociated. These investigators found that when $Mg(OH)_2$ was added to casein, 58 per cent of the total alkaline-earth metal was combined with the protein as a negatively charged complex ion; for $Ca(OH)_2$ 66 per cent; for $Sr(OH)_2$ some 54 per cent; and for $Ba(OH)_2$ 74 per cent.

Goigner and Pauli[27] studied the fixation of silver by serum albumin. These workers used a silver electrode and calculated the silver bound by means of the Nernst formula. Their results indicated that the amount of silver bound by the protein is not proportional to the concentration of the albumin. Through conductivity studies, these same investigators found that the amount of silver bound to the protein increases with increasing concentration of silver nitrate and tends toward a maximum value. Carroll and Hubbard[19] studied the influence of pH on the silver-binding power of gelatin. Their data indicate that the amount of Ag^+ ion bound increases with increasing pH value and the amount of Ag^+ bound is a minimum at the isoelectric point, but does not decrease to zero. At corresponding activities, gelatin was found to fix much more OH^- than Ag^+ ions.

Theis,[77] in an investigation of the neutral salt fixation by collagen, found that certain of these salts were bound to a greater extent than others. For this investigation Theis used a technique similar to that employed for the estimation of acid and base fixation. A great many experiments dealing with H^+ or OH^- ion binding had shown that such neutral salts as NaCl, KCl, and Na_2SO_4 have no significant influence on the isoionic point of the collagen; but such salts definitely influenced the slope of the titration curves. Theis' investigations on the collagen-neutral salt compound were confined to systems containing KCl, $CaCl_2$, and $BaCl_2$. Various neutral salt concentrations over a wide pH range were studied. Figure 31 illustrates the data taken for the systems [KCl-HCl and KCl-KOH] and [$CaCl_2$-HCl and $CaCl_2$-$Ca(OH)_2$]. The systems were varied from 0.1N to 1.0N with respect to neutral salt. This figure shows clearly that when 0.5N solutions of potassium chloride are used, no salt is bound at pH values lower than 5.0; when 0.75N solutions of potassium chloride are used, no salt is bound at pH values lower than 3.5; when 0.4N calcium chloride solutions are used, salt begins to bind at pH 3.0. The data further show that calcium chloride is bound much more readily than potassium chloride for any given concentration of salt solution. It was further found that maximum salt fixation occurred at the approximate isoionic point of the collagen-salt compound. All the curves show similar trends in that salt is bound over a wide pH range well into the alkaline

zone, the salt fixation decreasing to some extent at pH values greater than 10.0. Taking into account the large fixation of calcium chloride over a wide pH range and the shift of the isoionic point of the collagen-$CaCl_2$ compound,

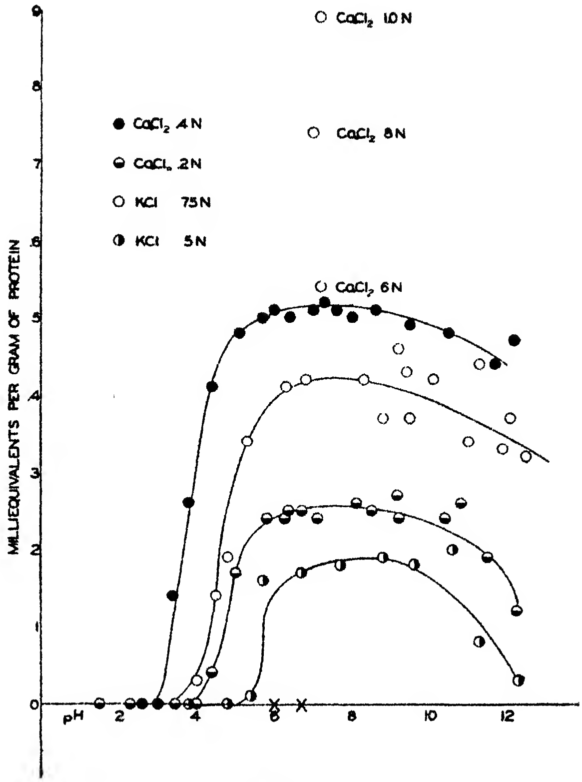


Figure 31

it would appear that the suggestions of Pauli and later of Leuthardt are substantiated; namely,

$$^{-}OOC—R—NH_3^{+} + Ca^{++}Cl_2 \longrightarrow {}^{-}OOC—R—NH_2 \cdot CaCl_2 + H^{+}.$$

These findings also substantiate the data given by Greenberg and Schmidt for calcium.

Using more concentrated solutions of calcium chloride (0.8*N* and 1.0*N*), it is found that the salt combined approximates the maximum acid fixation power of the collagen. The results obtained by Theis are somewhat at variance with those obtained by Northrop and Kunitz, in that Theis found KCl to fix with collagen.

Investigations upon the effect of $BaCl_2$ have shown results similar to those

of $CaCl_2$, and those employing Na_2SO_4 have given results essentially the same as those for KCl.

Theis and Jacoby found that the addition of such salts as KCl, NaCl, or Na_2SO_4 to acid or base solution in the determination of acid or base fixation had no effect on the isoionic point, but that they definitely influenced the slope of the titration curve. These investigators found, on the other hand, that the addition of $CaCl_2$, $BaCl_2$, or $HgCl_2$ not only affected the slope of the titration curve but caused a definite shift of the isoionic point to a more acid region. The binding of $CaCl_2$ at pH values as low as 3.0 undoubtedly influences the isoionic point of the collagen, and the $CaCl_2$-fixation must of necessity be the cause of the shift of this point to a more acid zone. If $CaCl_2$ binds itself to the amino groups as $-NH_3^+ + CaCl_2 \rightarrow -NH_2 \cdot CaCl_2 + H^+$, one would expect such a shift in the isoionic point.

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Chapter 5

Shrinkage Temperature of Collagen

In the early days of chrome tanning, the practical tanner applied a simple test to his leather—the boil test. This test was merely to place a piece of the chrome-tanned skin in boiling water and note whether curling took place. In this way the tanner judged the completeness of tannage, but no other significance at that time was attached to the test. Yet this test seems to hold immense possibilities in determining facts relating to the structure and the structural stability of the skin collagen.

History of the Shrinkage Temperature

As far as the authors can ascertain, Fahrion[13] in 1908 was the first investigator to discuss the behavior of animal skin in boiling water as a quantitative method of measuring "tanning." He mentions that under such treatment, ordinary hide powder is converted into glue, chamois leather is but little affected, and chrome-tanned leather is affected not at all. Ewald[12] in 1919 measured both the shrinkage temperature and the shrinkage percentage of connective tissue taken from mouse tails and frog leg sinews. He found that dilute HCl treatment decreased the shrinkage temperature and that the tanning procedure increased it. Later, in 1924, Powarnin and Aggeew[21] utilized such a test in estimating the actual temperature at which leather began to shrink. These workers studied the shrinkage temperature of a calf skin during its different stages of tanning, and found that after soaking it was 66.7° C, and after liming 42.2° C. They further pointed out that the shrinkage temperature might be made of real importance as a means of determining the degree of tannage. Schiaparelli and Careggio[22] in 1925 described a special apparatus for measuring the temperature of gelatinization (Tg). They made a number of measurements of chrome-, quinone- and vegetable-tanned skins, as well as delimed skins. They found the shrinkage temperatures to be 120, 79, and 45° C, respectively.

Chater[7] made use of this test, designing a special machine for more accurate measurement. Chater studied the effect of the stripping action of borax on vegetable-tanned sheep skin. Long immersion in borax solution decreased the shrinkage temperature. He also studied the effect of lime suspension upon calf skin and found that after 1 month the shrinkage temperature had decreased

from 65 to 47° C. Chater found that preliminary soaking in acid and alkali solutions gave shrinkage temperatures first increasing toward the neutral zone and then decreasing in the more alkaline zone. He correlated his data with swelling measurements and noted certain trends. In subsequent work, Chater[8] concluded that the actual shrinkage temperature would be an excellent index for detecting any departure from a usual tanning procedure. In 1930, Chater[9] studied the shrinkage temperature of beef tissue and found it to be approximately the same as that of beef skin. He found that certain iron salts or formaldehyde have an elevating influence upon the shrinkage temperature of skin. Later, in 1930, Chater summarized his various papers somewhat as follows. At pH 5 a special condition obtains in which the skin protein has the capacity of attaching a maximum amount of vegetable-tanning reagent. At this particular pH, the conversion of the skin into leather is as complete as it can be made, resulting in a product which has a definite initial shrinkage temperature, characteristic and maximum for the particular protein-tanning agent combinations.

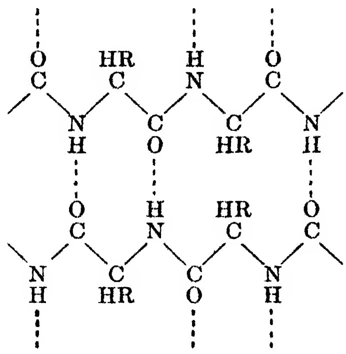
Chambard and Michallet[6] in 1927 made use of shrinkage temperature measurements in a comprehensive study of oil tannage, and used an apparatus similar to that employed by Schiaparelli. Casaburi and Corradini[5] in a study of the shrinkage temperature of various leathers concluded that it is characteristic for each type of leather. Theis and Schaffer[27] in 1936 designed a special apparatus for measuring the shrinkage temperature of either skin or leather. This apparatus, in reality a modification of that shown by Stiasny,[24] has since been approved and adopted by the American Leather Chemists Association. These workers made use of this instrument in their initial studies of formaldehyde tannage. Later, Theis and Kalb,[26] using this apparatus, studied the degree of leathering taking place during the chrome tanning process. Hobbs[15] in 1940 made use of this test in the study of vegetable-tanned leathers. Braybrooks[4] in 1939 made a rather extensive study of the relation of shrinkage temperature to pickling and tanning and suggested explanations of their findings. In some of the following chapters, certain data relating to the works of these writers will be further referred to.

The Theoretical Significance of Shrinkage Temperature

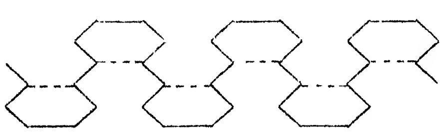
In previous chapters, we have discussed at length the structural chemistry of the fibrous proteins, and for this reason we shall now only point out the pertinent facts necessary for an adequate explanation of the phenomenon of the shrinkage temperature measurement.

According to the present accepted view of the structure of fibrous proteins, such as silk fibroin, hair or wool keratins, and skin collagen, the protein fibrils consist of micelles which are long in relation to their thickness. These micelles

are oriented parallel to the fiber axis. In the case of silk fibroin, this fiber pattern might be pictured thus:



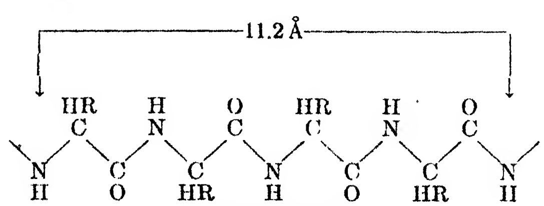
This diagram indicates that the polypeptide chains are held tightly together by means of coordinate bonds or hydrogen bridges between —CO—NH— groups in juxtaposition. Pauling and Niemann[20] point out that interactions of this type, while individually weak, can stabilize such huge structures as protein molecules by combining their forces hundreds of times in a polypeptide chain. As indicated in x-ray studies, silk fibroin is a fully extended protein structure; hence it does not show extensibility, but should show a certain contraction. Since silk fibroin contains but small amounts of basic and acidic amino acids, there can be but little doubt that the main stabilizing force is the Van der Waals or the coordinate forces pictured above. Astbury[1] and Speakman[23] have indicated that in keratin fibers the chains are probably folded into a series of pseudo-hexagons. These are shown in Figure 4 of Chapter 3. Such a structure might easily explain the contraction and extension of wool fibers. These fundamental researches indicate at least three types of cohesive forces within the keratin fiber; (1) covalent linkage —S—S—; (2) electrovalent or salt linkage $—COO^{-}{}^{+}H_3N—$; and (3) coordination of adjacent —CO—NH— groups of two chains. By stretching of wool fibers in the natural state (α-keratin) the looped backbone of the molecule is extended so that it corresponds to the backbone of the fully extended silk fibroin (β-keratin). Astbury believes such extension requires a breaking up of the disulfide or covalent linkage. Astbury and his colleagues have demonstrated a spontaneous contraction of the α-keratin molecule which they have termed "super-contraction." This type of structural change can be pictured:



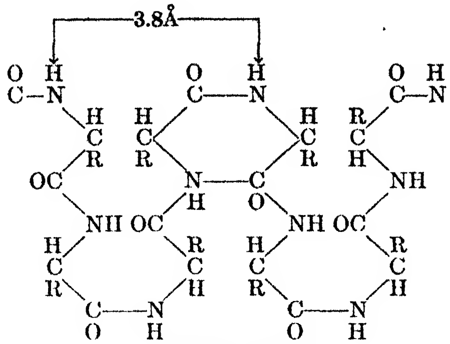
In α-keratin, the packing units are extremely close together and it is very difficult to penetrate them, as can readily be seen from hydration and swelling data.

In collagen, two types of cohesive forces must obtain; electrovalent or salt-like linkage and the coordinate or hydrogen bond. Thus, in collagen the bonding together of the polypeptide chains in all probability is largely the interaction between the numerous peptide linkages and to only a small extent between the charged centers. The collagen chains are not what might be called fully extended but are just slightly curled. The presence of bulky R— groups, such as arginine, lysine, proline or oxyproline in *cis* and *trans* configuration, may be responsible for the slight contraction of the collagen polypeptide chains. Astbury found through x-ray investigation 2.8 Å per amino acid residue in collagen in comparison to 3.5 Å per amino acid residue in silk fibroins.

In collagen, the packing of the individual units is not so close as is the case with fibroin and wool keratin. Again, its hydration is much greater, probably because of the charged active centers and free —CO—NH— groups. Collagen shows greater chemical reactivity than other fibrous proteins. The natural or native collagen is held in compact form by coordinate and electrovalent forces. Thermal agitation weakens these cohesive forces and the collagen tends to revert to a stable form not requiring a strictly parallel alignment of the primary chains. In a recent dissertation Astbury[2] points out "when the thermal agitation is sufficient to overcome the interchain attractions, the chain bundles may be said to melt and the chains collapse upon themselves. It follows that anything that interferes with the solution and interactions of the side chains that form the rings of the polypeptide grid, or with any inter-chain linkage for that matter, must inevitably influence the thermal transformation temperature." Astbury has further pointed out that coiling and uncoiling of peptide chains is very common in proteins. He has shown by x-ray studies that globular proteins are coiled and extend upon denaturing, that wool and keratin are partly coiled and are capable of both extension and contraction, but that silk fibroin and collagen are fully extended and are capable only of contraction. This contraction can be pictured somewhat as has been suggested by Gustavson:[14]



Natural Collagen



Shrunk Collagen

In native collagen, the coordinate valences are in all probability available for the binding of water or for interchain reaction. Upon thermal shrinkage, however, an initial compensation of these groupings may take place as shown above. This molecular rearrangement shows striking similarities to the melting of gelatin jellies.

Braybrooks, McCandlish and Atkin[4] have discussed in detail the relation of swelling to the shrinkage temperature of collagen. They point out the following pertinent facts regarding their research: osmotic swelling is the result of osmotic pressure generated by an excess of diffusible ions within the protein and a simultaneous reduction in cohesive forces caused by the back titration of the zwitterions; at extreme pH values, or in the presence of an increasing concentration of dissolved ions, the osmotic pressure decreases but the reduction in cohesion usually still obtains; swelling curves show the osmotic pressure generated by the free carboxyl groups at pH 6 and it is maintained that the back titration of histidine from pH 4 to 8 should lead to a small reduction in shrinkage temperature; lyotropic swelling is the result of a reduction in cohesion without the production of a swelling pressure. Braybrooks *et al.* believe that the shrinkage temperature is largely dependent upon the zwitterions, which can be broken by back titration.

It should be pointed out that the chromium salt added during chrome tannage must also affect the zwitterion structure of the protein, but in this case the shrinkage temperature of the collagen is increased. They also postulate that the short links are acted upon and broken by the more active neutral salts. In a recent publication, Wilson[29] points out that the so-called hydrogen bond is extremely stable under ordinary conditions; but when the protein becomes unduly swollen by solutions of acids or alkalies, or by the application of hot water, rupture of the hydrogen bond occurs and the initially stable structure is lost. He further points out that the instability of the structure of raw protein in contact with boiling water is due to the weakness of cross links.

Pauling,[19] in a recent paper dealing with a suggested theory of the structure and process of the formation of antibodies, utilizes the modern theory of long-chain molecules held together by the hydrogen bond linkage and then develops a theory of the structure and *modus operandi* of antibody formation and the chemical nature of serological reactions which is more definite and more reasonable than many earlier theories. Pauling has suggested that all antibody molecules contain the same polypeptide chains as normal globulin and differ from it only in configuration of the chain or, in other words, in the manner in which the chain is coiled. Braybrooks has shown that the peptide chain in collagen is held in the extended condition, but that it will coil upon removal of the restraining forces or structural cohesion. Since rise in temperature increases the vibrational energy of the molecule, it would be natural, then, for the collagen fibers to contract upon heating, because of the reduction of cohesion due to the ionic attraction of the zwitterions and to the short link.

Thus the shrinkage temperature of a protein may be defined as the point at which the increasing disruptive tendencies exceed the diminishing cohesive forces, thus making the shrinkage temperature actually a measure of the structural stability of the collagen expressed in temperature units.

In this chapter, the authors will use frequently the term "hydrogen bond" or "bridge," but they must of necessity point out that the hydrogen bond is still somewhat a matter of speculation in protein structures—a fact which must be borne in mind. Astbury[3] has stressed this point in a recent lecture, "As things stand at the moment, the question of the hydrogen bond in protein structure is not at all one on which to embark with any confidence. To put the matter frankly, the detailed structure of the proteins is still largely unknown, and therefore, *a fortiori*, the role, if any, played by the hydrogen bond in protein structure is also largely unknown." Whenever the authors have used the term "hydrogen bond" in the work to follow, they have merely taken advantage of current knowledge to bring about clarity of interpretation of their work. The reader may prefer the equally vague term "Van der Waals forces."

The Effect of Acid, Base and Sodium Chloride on Shrinkage Temperature

In collagen, we should expect the salt linkage and the hydrogen bridge to be the cohesive forces most prominent in contributing to the structural stability of the protein. By means of the shrinkage temperature, we should be able to differentiate the effect of each of these two linkages. Figure 32[28] indicates the structural stability, as measured by the shrinkage temperature, over the pH range 1 to 13.5, with and without salt addition. Curve A, representing acid or base solutions without added salt, indicates that mild alkali (pH 7 to 10) has little or no effect upon the structural stability of the protein, but that acid (pH 4 to 1) has a very decided effect. At pH values

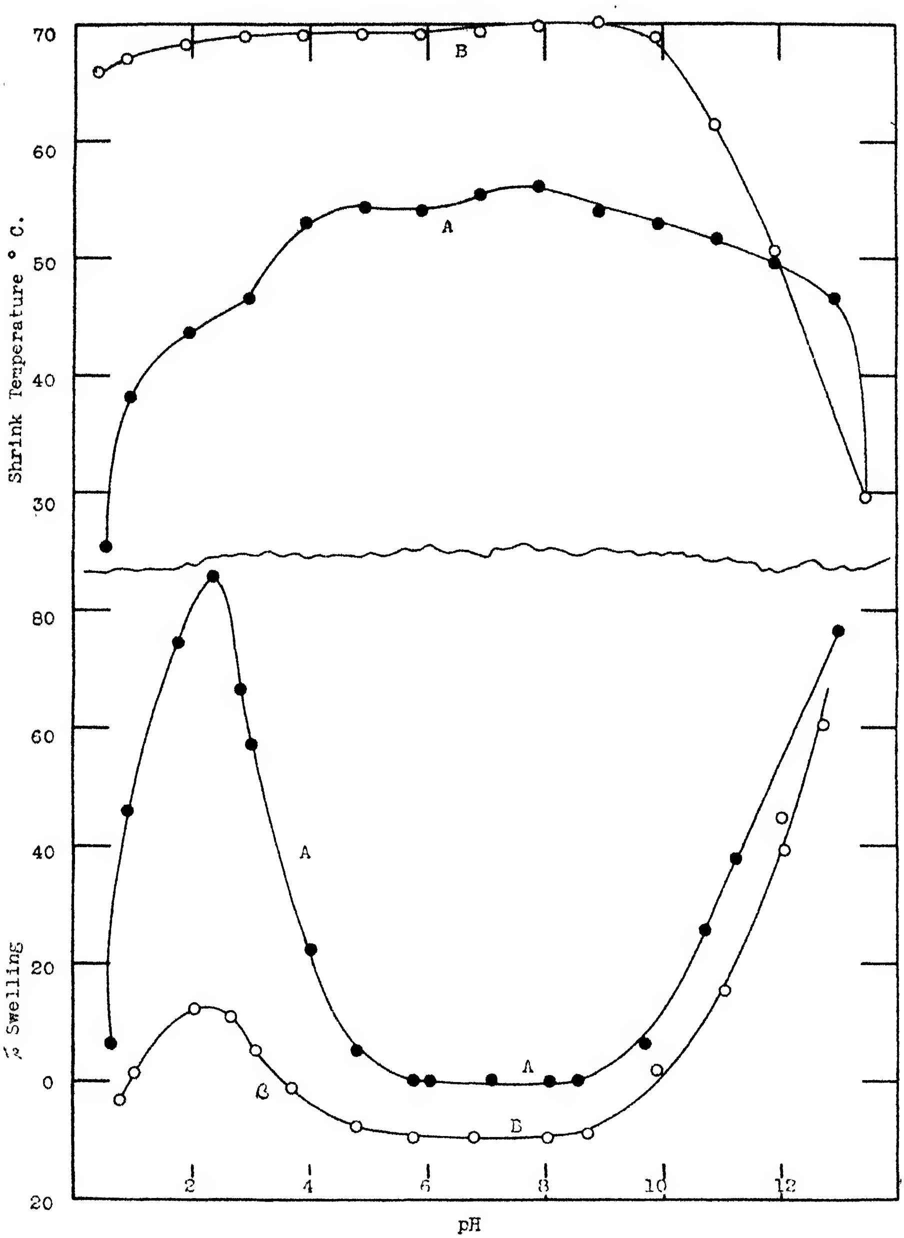


Figure 32. Relation of swelling to shrinkage temperature of collagen treated at various pH values with water or sodium chloride solution. Taken from data of Theis and Steinhardt.

greater than 12, a definite decrease in stability results, indicating structural breakdown in this region.

From these facts, it is rather evident that the action of the acid in the low pH range and the alkali in the very high range is upon the short link or hydrogen bridge. If we examine the curve closely, we can visualize certain probable chemical reactions taking place. In the pH range of the isoelectric point up to 9, a back titration of histidine in all probability occurs. However, since the amount of histidine is small, the effect of such back titration should not be great. As the pH value increases to 12, any lysine should be affected, and at pH values greater than 12, the guanidino group of arginine should be titrated. On the acid side of the isoelectric point, the aspartic and glutamic acid should be affected, being back titrated and probably reaching approximate maximum values at pH 2. Thus, we might say in the pH range 2-13, salt linkages are affected—the effect being dependent upon pH value. The breakdown of structural stability due to salt linkage might be pictured as follows:

$$\vert\!-NH_2\quad HOOC-\!\vert \xleftarrow{\text{acid}} \vert\!-NH_3^+\quad {}^-OOC-\!\vert \xrightarrow{\text{alkali}} \vert\!-NH_2\quad {}^-OOC-\!\vert$$

Further consideration of Curve A and values representing acid-base fixation indicate that at pH 3 approximately 66 per cent of the acid-fixing groups have been satisfied- indicative of a reasonable destruction of salt linkages. This same reasoning will obtain at pH 12. Thus it would be logical to assume in the pH range 3 to 12 that most of the changes in structural stability are due to effects upon the salt linkage. There are undoubtedly effects upon the hydrogen bond linkages in this region also, and these will be discussed later. Curve A indicates that the back titration of the charged amino or carboxyl groups affects the stability of the protein to a very reasonable degree only.

The effect of strong acid or alkali upon the hydrogen bond is especially noticeable at pH 1 and 13. However, the structural stability of the protein due to the hydrogen bond linkage is affected in the acid range 1 to 3 and alkaline range pH 12-14 as can readily be seen from the curve in those regions. In the pH range 3 to 1, Curve A shows a decided break in structural stability indicative of a more drastic change than the mere back titration of charged carboxyl groups $\vert\!-NH_3^+\ {}^-OOC-\!\vert \rightarrow \vert\!-NH_3^+\ HOOC-\!\vert$, since a goodly number of these salt bridges must have been weakened and broken in the less acid region. It would therefore appear that acid, especially at low pH values, has a real and pronounced effect upon the short link or hydrogen bond structure. In the pH range 2 to 3, a large amount of swelling occurs, which undoubtedly affects the ability of the structure CO—NH to maintain itself, and thereby structural weakness occurs. At pH 1 swelling again reaches a

comparatively low value, as shown by Figure 32; but even though osmotic pressure has decreased, the reduction in cohesion is still evident. This fact was also pointed out by Braybrooks *et al.*[4] Curve A of Figure 32 strongly indicates that shrinkage temperature measurements will allow differentiation of action upon the salt or hydrogen linkage.

Curve B of Figure 32 shows the shrinkage temperature of skin treated with salt-acid and salt-base solutions over the pH range 1 to 13. All the solutions used were 2.0*N* with respect to sodium chloride. Comparing Curve B with that of Curve A, it is readily seen that the salt additions cause a real increase in the shrinkage temperature of the treated skin. This increased shrinkage temperature is noted only in the pH range 1 to 10, indicating that the pickling effect occurs only in this range and is not effective in strong alkaline solution.

A careful study of Curve B gives rise to further possibilities in the matter of protein structural stabilization. In the first place, the sodium chloride is instrumental in decreasing swelling over the entire pH range, but especially from pH 1 to 4 and 6 to 10. In solutions having a pH greater than 10, the sodium chloride is not able to control this swelling factor. The reduction in swelling should make for a closer fibril network and thus cause a strengthening of the cohesive forces. That such is the case can readily be seen by the great increase of shrinkage temperature in the pH ranges 1 to 4 and 8 to 10. Therefore, in the very acid range, the decreased swelling and dehydration might readily strengthen the hydrogen bond type of linkage. In the very acid range (pH 0.5 to 2.0) complete breakdown of salt linkages would have occurred even in the presence of the sodium chloride. Such weakening of this type of bond does not affect materially the structural stability of the protein. Curve B demonstrates this fact rather conclusively in the range pH 0.5 to 4.0.

Such facts as these bear out the statement that dehydration within reason causes greater structural stability, whereas swelling has the reverse effect. Curve B further substantiates the contention that it is the effect upon the $>$CO- - - -NH$<$ linkages and not on the salt linkages that really brings about structural weakness. While it is true that there is a weakening of cohesion forces through back titration of charged groups in the acid and alkaline range, the actual breakdown in structure in the very acid and very alkaline ranges is undoubtedly due to action of the strong acid or base upon the hydrogen bonds. The addition of salt (NaCl) in sufficient amounts retards such action and through dehydration actually strengthens the cohesion forces, as can be seen from the shrinkage-temperature measurements.

Figure 32 shows the ability of a 2*N* solution of sodium chloride to strengthen the cohesion forces of collagen over the pH range 0.5 to 10.0. Braybrooks *et al.* postulate that sodium chloride causes a reduction in shrink-

age temperature. These workers, however, used a $0.1N$ solution, and point out from a swelling curve for gelatin that this idea is in line with swelling phenomena. The gelatin swelling curve is taken from the work of Jordan-Lloyd,[16] wherein she shows actual lyotropic swelling by $2N$ NaCl at the isoelectric point. However, in a later curve using collagen material (goat skin) Jordan-Lloyd[17] shows actual dehydration at the isoelectric point. The swelling curve given in Figure 32 shows less swelling over the entire pH range when using collagen strips and acid-base-salt solution made $2N$ with respect to sodium chloride. Thus for the collagen material used, the shrinkage temperature and swelling data are well correlated.

Effect of Mono- and Divalent Salts

When less than $2N$ concentrations of NaCl are employed, the results differ from those given above. Figure 33,[28] Curve D, shows the effect of concentrations of sodium chloride varying from zero to $3N$, all solutions being approximately pH 5.5 with respect to hydrogen ions. This curve shows that dilute sodium chloride solutions actually cause a reduction in cohesion forces—an action undoubtedly upon zwitterions or upon salt bridges—perhaps pictured as suggested by Braybrooks:

$$
\begin{array}{|llllll|}
Cl^- & Cl^- & & & Na^+ & Na^+ \\
\text{---}NH_3^+ & Cl^- & Na^+ & {}^-OOC\text{---} & & \\
Cl^- & Cl^- & & & Na^+ & Na^+
\end{array}
$$

Such reduction in cohesion forces is small as measured by the reduction in shrinkage temperature of some 5° C. Curve D shows, however, that at a salt concentration of 1.5 moles, no reductions of cohesion result, *i.e.*, the breakdown in salt linkage is exactly counterbalanced by strengthening of the hydrogen bonds by dehydration caused by the salt at this particular concentration. However, as the salt concentration rises above $1.5M$, the increase in cohesion forces (hydrogen bonds) due to subsequent dehydration is much greater than the reduction in cohesion forces caused by the action of the salt on the salt linkages.

It has been noted from time to time that skin actually shrinks at temperatures lower than 20° C in concentrated solutions of calcium chloride.[4,14,28] This phenomenon has been carefully studied. The skin was placed in the shrinkage meter and the medium of heat exchange used was a solution of calcium chloride, the concentration of salt varying over a wide range. In this way Theis and Steinhardt[28] were able to study the immediate effect of the calcium chloride solutions. Curve A of Figure 33 gives the data so obtained. Curve B of this figure shows similar data, but in this case the skin was allowed

to remain in contact with the calcium chloride solution for 48 hours before determination of shrinkage temperature. In the latter case, it was found that the skin actually shrank, at the room temperature prevailing, at a concentration greater than 1.2*M*.

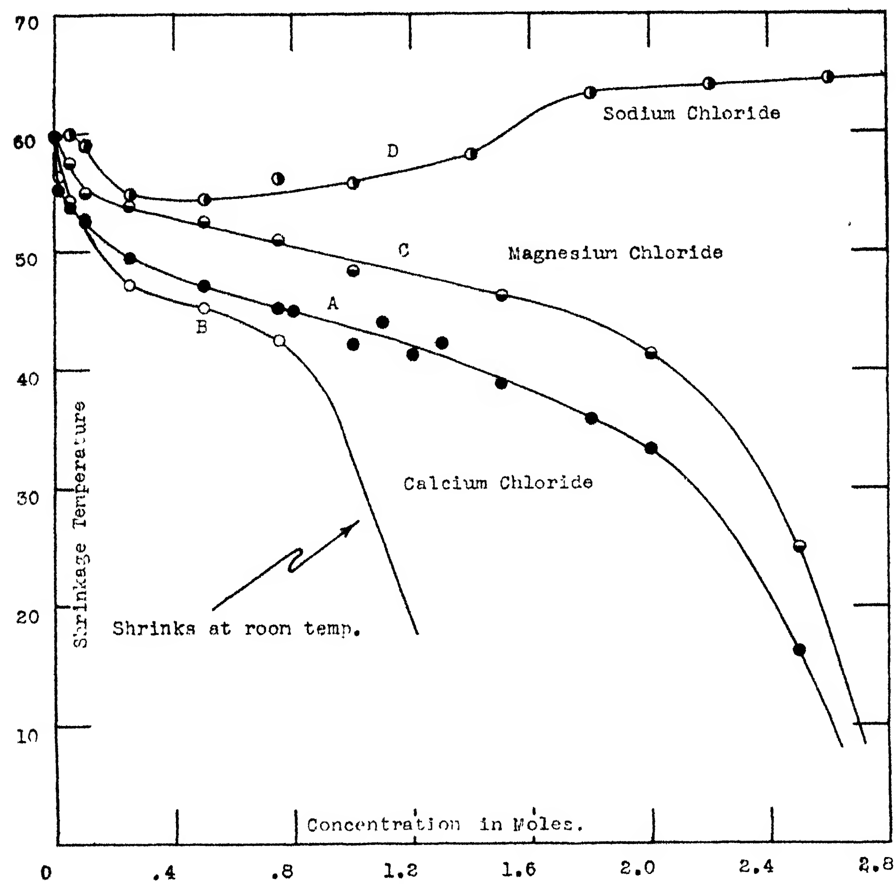


Figure 33. Shrink temperature of collagen treated with various salt solutions and various concentrations of salts. Taken from the data of Theis and Steinhardt.

Curve A shows that a calcium chloride concentration of 2.6 moles causes instantaneous shrinkage at 16° C. Comparing Curves A and B with D (NaCl over same concentration range), it is seen that the effect of calcium chloride is entirely different from that of sodium chloride. Since the data for each of these curves were obtained at approximately pH 6, *i.e.*, in the isoelectric zone, the effect of the neutral salts should be upon the zwitterion salt linkage and

in the region of lyotropic swelling. Curve B shows conclusively that calcium chloride, even in concentrations as low as $0.01M$, has a real effect upon the structural stability of the protein. Although sodium chloride in the concentration range 0.1 to $1.4M$ decreases the shrinkage temperature at most 5° C and at $1.6M$ actually increases this unit some 2° C, calcium chloride in this same concentration range decreases the shrinkage temperature some 20° C (concentration $1.6M$).

Sodium chloride at concentrations greater than $1.5M$ actually increases the cohesion forces of the protein; we therefore are inclined to picture calcium chloride as having an entirely different effect upon the collagen structure from that of sodium chloride. It appears to the authors that sodium chloride has two different and distinct actions upon collagen structure, depending upon the concentration. In dilute solution it acts only on the salt linkage; but in concentrated solution it not only acts on the salt linkage, but due to actual dehydration and withdrawal of water from the protein, it causes a strengthening of the existing short linkages, thus creating a net gain in cohesion forces.

The particular action of calcium chloride is evident in both dilute and concentrated solutions. Calcium chloride affects both the short link and the salt link. In more or less dilute solutions, the effect appears to be upon the salt linkage, causing a weakening of this type of structure. In this respect it appears to be far more effective than sodium chloride. It must be remembered that calcium chloride actually causes swelling (pseudo) in the isoelectric zone and also has an extreme peptizing effect upon collagen. Thomas measured this effect in 1925[28A] and showed that the degree of hydrolysis of collagen with calcium chloride was some three times greater than with sodium chloride. Thus calcium chloride does not have the dehydrating effect of sodium chloride and actually gives a swelling effect, thus placing a strain upon both the salt and short linkages. At concentrations greater than 0.8 mole of calcium chloride, the effect must of necessity be mainly upon the hydrogen bonds, since complete breakdown of salt linkage could not cause such reduction in cohesion forces (see Figure 32, Curves A and B). Curve B, Figure 33 illustrates the breakdown in each type of linkage.

Curve C of Figure 33 shows the data using magnesium chloride in place of calcium chloride. It indicates the same general trend, *i.e.*, magnesium chloride causes a breakdown in both types of linkage within the collagen molecule. The breakdown is not nearly as drastic as that caused by calcium chloride; but the specific action, both on the salt linkages and on the hydrogen bond, is strikingly indicated.

The foregoing data have shown the effect of some neutral salts on the structural stability of collagen. Table 28 gives data showing the shrinkage temperature of collagen when treated with various other reagents. It shows that all acids have a similar effect on the structural stability, the effect pro-

Table 28.

Reagent	pH	Shrinkage Temperature (°C)
H_2O	4.9	57.0
HCHO	8.0	83.5
Picric acid	1.82	50.8
Phosphotungstic acid	3.00	45.0
Tannic acid	4.57	74.5
0.1*N* HCl	1.3	42.2
0.1*N* CCl_3COOH	1.3	39.7
0.1*N* H_2SO_4	1.18	40.2
0.1*N* acetic acid	2.9	48.9
0.1*N* HCl + NaCl	1.1	62.7
0.1*N* H_2SO_4 + NaCl	0.9	63.9
0.1*N* H_2SO_4 + Na_2SO_4	1.6	68.2
0.1*N* NaOH	13.0	45.8
0.1*N* NaOH + NaCl	13.0	37.8
N HCl + NaCl	0.4	63.1
2*N* HCl + NaCl	0.35	65.3
2*N* $CuCl_2$	—	45.2
2*N* $ZnCl_2$	—	49.7

duced being more or less dependent upon the equilibrium pH value obtained. Similar effects are noted for alkalies.

The foregoing pages have stressed the effects of mono- and divalent salts upon the so-called salt bridges and hydrogen links in the structural make-up of collagen. It was pointed out that the swelling in the isoelectric zone is in the main responsible for the drastic effects of calcium and magnesium chlorides. That this swelling exists is plainly shown by the work of Jordan-Lloyd,[17] Braybrooks,[4] and Northrop and Kunitz.[18] These last workers showed rather conclusively that dilute solutions of sodium and potassium chloride increase swelling, but that at concentrations greater than 1.8*N* dehydration begins; that calcium and magnesium chlorides increase swelling more and more as concentration of salt increases until complete solution takes place; and that aluminum chloride causes swelling in dilute solution, but dehydration in more concentrated solutions. The work of Northrop and Kunitz well supports our rather dogmatic conception of dilute neutral salts affecting the salt linkage, while concentrated divalent salts decrease the cohesion forces due to the hydrogen bonds. Figure 34, taken from the work of Northrop and Kunitz,[18] shows almost identical correlation between swelling and our measure of cohesion forces. Their swelling curves for calcium and magnesium chlorides in reality indicate in a different manner the actual breakdown in structure; but for sodium chloride the hydration and dehydration portions of the swelling curve indicate the postulated two effects of dilute and concentrated solutions upon the structural stability of collagen. The correlation between the swelling curves, osmotic-pressure development and the shrinkage-temperature measurements is, from the authors' viewpoint, remarkable and of extreme practical significance.

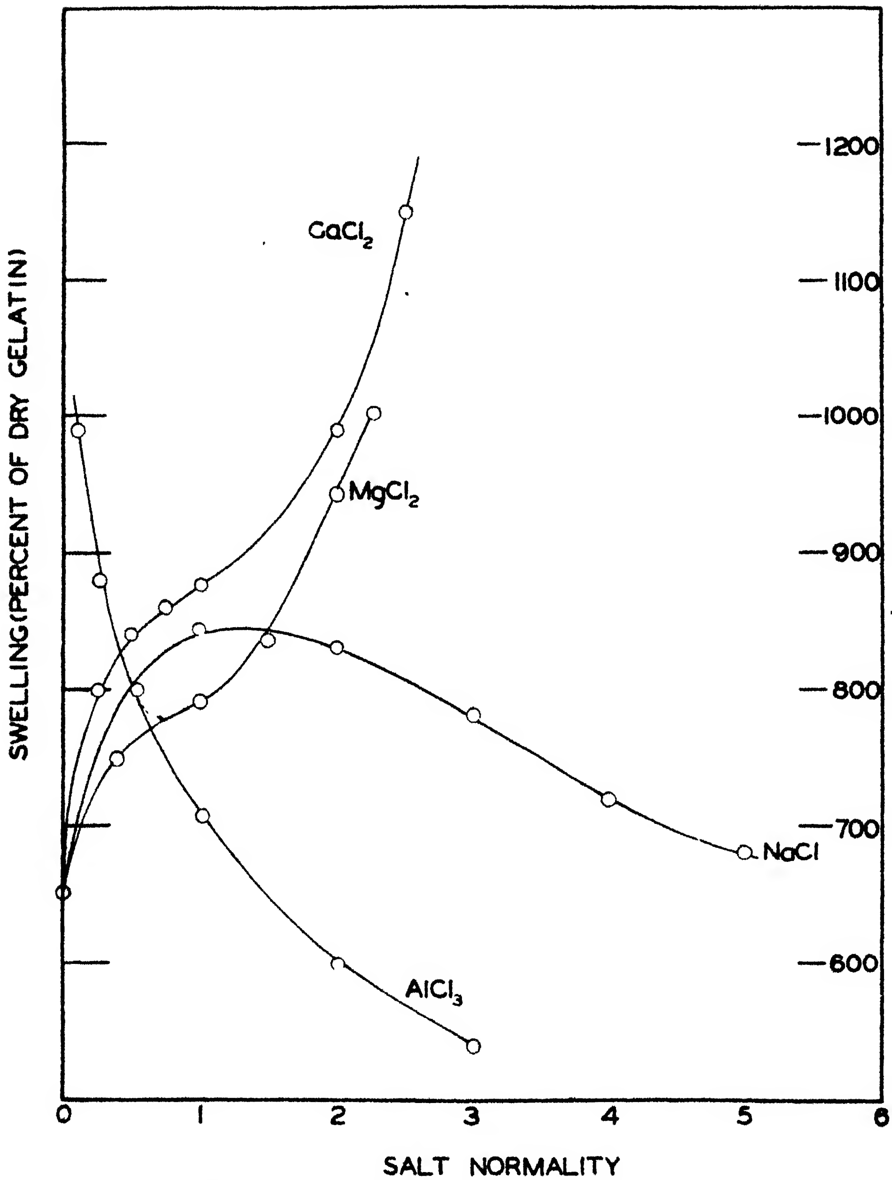


Figure 34. Effect of concentration of various salts upon swelling of gelatin.

So far in this chapter, the authors have dealt only with the relation of shrinkage temperature to the structural forces inherent in native collagen. This relation is of very definite importance in liming, pickling and tanning. Though the shrinkage temperature measurement of leathers will be treated in detail under specific headings later in this treatise, some mention of its possibilities are herein included for clarity.

It is appreciated that many good commercial leathers are unable to stand the boiling test, but it is found that the properties of many leathers are changed by carrying on the tanning operations so as to increase the temperature at which they shrink when immersed in hot water. This illustrates the importance of measuring the "shrinkage temperature" of leather. For example, in chrome tanning it is often found that fixing more chrome in the leather by additional neutralization raises the shrinkage temperature. Tanners of shoe-upper leathers find it desirable to control the shrinkage temperature of leather during tanning to obtain the properties best for the purposes for which the leather is to be used. In many cases it is found that the best shrinkage temperature is at a point slightly below the boiling point of water.

Gradually it has been learned that the shrinkage temperature of vegetable-tanned leather is as important as that of chrome-tanned leather. It is found that the shrinkage temperature of leather is a more reliable guide to the extent to which true tanning has taken place than is the measurement of "combined" tanning material.

Measurement of the Shrinkage Temperature of Leather

In 1941 a method[11] was proposed for measuring the shrinkage temperature of leather, and it is given below as published:

Definition: When leather is gradually heated in an aqueous medium, a temperature will be found at which noticeable shrinkage occurs. This is defined as the shrinkage temperature.

Scope: The method is designed to measure the shrinkage temperature of all leathers at any point in the tanning process.

Each sample shall be 0.5 inch wide by 3 inches long. The full thickness of the leather is used. No conditioning is necessary.

Apparatus: A 1-liter beaker containing 75 per cent glycerin in water (sp. gr. 1.190), two clamps for holding the sample, a stirrer, a thermometer and a heating device. The clamps are mounted vertically, 2.5 inches apart with the stationary one at the bottom. To the upper or movable clamp is attached an indicating device which will maintain the sample under slight tension, which will indicate any preliminary swelling in the sample and which will accurately detect the point at which shrinkage begins. This shall magnify the movement of the leather by at least 25 times.

Procedure: Place the sample to be tested in the clamps, completely immerse in the glycerin solution at room temperature, and heat the solution, with stirring, at the rate of 3 to 5° C per minute. As the temperature rises, swelling of the sample occurs and the indicator may be adjusted to a reference or zero point so that eventual shrinkage can be readily detected.

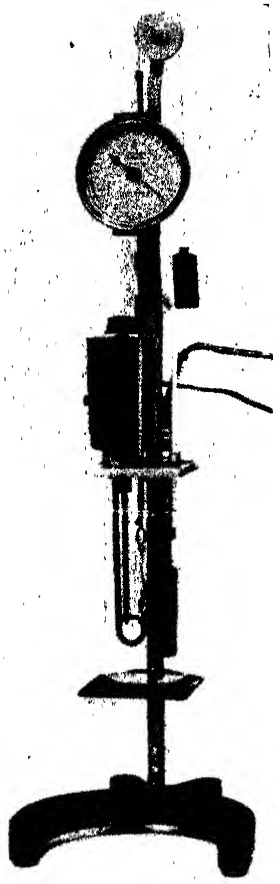


Figure 35. Theis Apparatus for the Measurement of the Shrinkage Temperature of Raw Skin and Collagen

In addition to the weight necessary to counterbalance the weight of the clamp or to overcome the inertia or friction of the indicating devices, it will be found advisable to add an additional weight of from 80 to 100 grams. The smaller weight will suffice for lightly vegetable-tanned leather such as sheep skin, and the larger weight is advisable for heavy leather such as chrome-tanned side leather. In no case should a weight be used which will cause an elongation, prior to shrinkage, of more than 10 per cent.

Report: Record the temperature of the bath in degrees centigrade at which the sample begins to shrink after the preliminary swelling.

When the great importance of measuring the shrinkage temperature of leather became recognized, it was natural that precision equipment should be developed for making this measurement. Some tanners merely place a strip of leather of known area into a beaker of water and raise the temperature slowly, noting with each degree rise in temperature whether the area of the leather strip has decreased. The lowest temperature at which measurable shrinkage is noted is taken as the shrinkage temperature.

Theis[25] has developed an apparatus for making this measurement which is not only simpler to operate but is also capable of much greater precision. This apparatus is illustrated in Figure 35. The strip of leather is clamped into place between the jaws provided, and then the water or glycerin solution is placed on the movable platform so that the leather strip is completely immersed. An automatic stirrer is provided to keep the solution thoroughly agitated. By means of a heating device, the solution is heated very slowly and a thermometer is provided to show the temperature at all times. At the shrinkage temperature of the leather, it begins to contract and the extent of shrinkage is measured by the number to the right of the hand on the dial. In this way measurements of shrinkage temperature can be made within less than 1° C. Glycerin solutions may be used instead of pure water when measurements at temperatures below 0° C and above 100° C are desired.

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Chapter 6

Microörganisms

The control of microörganisms constitutes a major problem in tanning. This problem will be considered in detail when we discuss tannery processes in which microörganisms are involved. We deal in this chapter with the more general description of microörganisms and with their behavior.

There are four general divisions of plant life: *Spermatophyta* (seed plants), *Bryophyta* (moss plants), *Pteridophyta* (fern plants), and *Thallophyta*. The last division is one of simple plants which is never differentiated into roots, stems, or leaves. It includes bacteria, yeasts, and molds and is therefore the division which is important to our subject. The *Thallophyta* may be divided into three groups: the *Schizophyta* (chlorophyll-containing algae); *Schizomycetes* (bacteria, or unicellular plants, containing no chlorophyll and multiplying by cell fission only); and *Fungi* (unicellular or multicellular plants, containing no chlorophyll, multiplying by means other than fission, and including yeasts and molds).

Bacteria

The most important microörganisms in tanning are bacteria. These are usually classified, on a morphological basis, into three general groups: a *coccus* is a spherical cell, a *bacillus* a straight rod, and a *spirrillum* a bent rod. As is well known, bacteria are very small, ranging from 0.5 to 10.0 microns in length. They multiply by a process termed fission; that is, the bacterial cell grows until it has practically doubled in size, and it then separates into two individual cells. These cells may or may not become detached from each other; sometimes they cling together and form characteristic groups. When, for example, the *cocci* divide and form long chains, they are termed *streptococci*; when irregular clusters form, they are called *staphylococci*; and when they unite in pairs, they are designated as *diplococci*.

Some bacteria possess thread-like organs of locomotion known as *flagella*. Bacteria multiply with great rapidity under conditions favorable to their growth. This subject will be dealt with later.

Certain bacteria have the power of producing spores during their growth, and these spores are usually much more resistant to destructive forces, such as heat, than are the parent cells. The spore form appears to be a method of protection against unfavorable environment.

The two most important general groups of bacteria in tanning are termed "proteolytic" and "non-proteolytic." As these terms imply, the first group digests protein, while the second does not, or at least to a very slight extent. Food necessary for bacterial growth must diffuse into the bacterium through its cell wall. Insoluble protein obviously cannot diffuse through the cell wall, but the proteolytic bacteria secrete enzymes which attack the protein, rendering it diffusible. Thus when we speak of bacterial digestion of protein, for example, we mean that the protein is digested by bacterial enzymes.

Bacterial growth and activity are governed by numerous factors, such as available moisture, reaction of environment, kind of food available, temperature, and gaseous environment. And these factors are usually interdependent, as will be seen as we proceed.

Methods for bacterial identification and for making bacterial counts are given in standard bacteriological textbooks. The latter subject has been especially discussed in its relation to tanning studies by Wilson.[3]

Yeasts

Yeasts are single cell organisms; their cells are usually larger than those of bacteria, and they reproduce themselves by a process of budding, except in the case of the genus *Schizosaccharomyces* which reproduces by fission. Yeasts are of theoretical interest in tanning because of their well known power of fermenting sugars. A felt-like covering is sometimes observed on old tan liquors, and this is said to be a yeast, *Saccharomycetes mycoderma.*

Molds

Molds differ from bacteria and yeasts in that they are multicellular and that their method of reproduction is more complicated. They consist of two kinds of cells, the assimilative and the productive. As a mold grows it forms a mass of branching threads which has the appearance of a cobweb. There are tiny projections along these threads from which the spores develop. The main mass of threads is termed the *mycelium*, and an individual thread is called a *hypha.*

There is an infinite number of species of molds, but the common molds have been divided into five general families: (1) *Mucoraceae*, (2) *Mucedinaceae*, (3) *Dematiaceae*, (4) *Stilbaceae*, and (5) *Tuberculariaceae.*

Both the morphology and physiology of molds have been discussed at length by Doelger and McLaughlin,[2] and by Wilson[3] in the second edition of this monograph, to which the interested reader is referred. But it may be stated that the majority of molds with which the tanner is concerned belong to two groups: the *Penicillia* and the *Aspergilli*, both of which are included in the *Mucedinaceae* family.

Molds are frequently present in vegetable tan liquors and may cause

destruction of tannin. It will be noted in Chapter 17 that the early observation of the botanist Van Tieghem of the production of gallic acid when tannic acid is acted upon by *Aspergillus niger* constitutes one of the foundations of our knowledge of the organic chemistry of the tannins. An acid environment is most favorable to mold growth, and an alkaline environment is unfavorable. It is therefore not surprising to find mold growing on pickled skins, from which Blank[1] has isolated mold growing at a pH value below 1.0. Wet chrome leather may become moldy during any of the stages following tanning and develop "mold spots" which cannot be removed, and which appear in the finished leather as discolored areas. Moreover, mold is often present in the rooms or lofts in which heavy vegetable leather is dried, and this infects the leather.

The prevention of mold growth is a constant problem to the tanner, since the atmosphere is usually heavily laden with mold spores. The only successful preventive measure, in addition to cleanliness of rooms and equipment, is the employment of the proper kind and amount of fungicide. There are many satisfactory fungicides on the market.

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Chapter 7

Curing

Animal skin is covered with countless bacteria, the amount and kind varying with the environment of the animal and with the quantity and nature of filth on the skin. During life these bacteria have little or no effect upon the skin of the animal. But as soon as the animal is slaughtered and life processes cease, the proteolytic bacteria may and do quickly attack the skin. The value of the skin to the packer, to the tanner, and to the public is in direct relation to the extent of its digestion by bacteria and, to a less extent, to degenerative changes other than bacterial. To arrest these degenerative processes, the producer of the skin "cures" it. That is, he partially dehydrates the skin by treatment with salt, such as sodium chloride, or by drying it in the air, or by a combination of the two methods. Bacteria require free water for their metabolism. One hundred pounds of fresh steer hide contains some 62 pounds of water, part of which is free and part chemically combined with the skin proteins. The dehydration methods referred to reduce or eliminate the available or free water.

Great care is taken in the better packing houses to cure and preserve the skin properly, since it represents a very considerable proportion of the total selling value of the processed animal. We will therefore briefly describe the practice of such packers, which may be termed the American standard.

In the case of steers, bulls, or cows, the animal is stunned by a blow on the forehead with a sledge hammer. It is then hoisted to a vertical position head down, by means of a chain which is attached to one or both hind legs. After this, the jugular vein is severed with a sharp knife, and the animal is bled. The hide is then flayed from the carcass and is immediately dropped down a chute to the curing cellar floor. The temperature of the curing cellar is about 13° C. The warm hide lays for about two hours in order that its body heat may escape, since putting a warm hide into the curing pack would encourage the very bacterial action which is to be arrested. The packer is very careful, however, not to delay curing after the hide has cooled, since bacterial action may actually start before the hide is completely cooled. The cooled hide is then spread out, flesh up, on a layer of clean rock salt and is covered with salt. Another hide (flesh up) is laid on top of the first hide and is salted, and this process is continued until a "pack" has been completed. The size and shape of the pack may vary, but the general rule is

that its length shall be about twice its width, or, say, 20 by 40 feet in area and 4 feet high. The pack is arranged so there will be proper drainage of the excess of the salt brine which is formed and of the brine-dissolved blood, lymph, and other soluble proteins. This method of curing requires a minimum of three or four weeks in pack, at which time the original fresh hide will have lost part of its moisture and will have gained weight through the salt it has absorbed. The algebraic sum of these two values shows that 100 pounds of original fresh hide weighs about 80 pounds in the salted or cured condition; this weight loss is termed the "shrink."

A better curing method for heavy hides is generally practiced in the *Frigorifico* establishments of Argentina and is as follows: The animal is thoroughly washed before slaughter to remove the bulk of surface filth. The flayed hide is again washed, both flesh and hair sides, and the flesh side is vigorously swept with a broom. The washed hide is drained and is then placed in a cool saturated sodium chloride brine for 24 to 48 hours, with occasional stirring. The hide is then removed from the brine, drained, and salted down in packs as described above. This method will be discussed at length later on in this chapter.

Calves are not stunned, but are shackled by a hind leg and hoisted to a vertical position; the jugular vein and other neck blood vessels are then severed, and the animal is thoroughly bled. This operation requires about six minutes. After this, the carcass is thoroughly washed, and is then placed in a cooler at 1° to 2° C for 24 hours before flaying. The flayed skin is then salted down in packs for about 30 days.

Many imported hides and skins are not salt-cured, but merely air-dried in those countries where salt is not available or where drying is preferred. Such hides are termed *flint*, or *flint-dried*. This stock may contain as little as 8 per cent moisture. When heavy hides are dried, a considerable amount of decomposition may occur; if they are dried too slowly, bacterial action ensues, and if too rapidly the surfaces are dehydrated; but the interior is still wet and is therefore liable to putrefaction. Dried heavy hides often show bacterial damage which is usually not apparent until they have been partially processed in the tannery. The soaking of such hides presents a special problem to the tanner which will be discussed in Chapter 8.

Probably 99 per cent of all the goat and kid skins used in the United States are imported, mainly from Asia, Africa, and South America. The great bulk of these skins are in the dry state, some having been cured by simple drying and others by salting prior to drying, in which case they are termed *dry-salted*. In many countries which produce dry-salted skins sodium chloride is not available, and the curing salt is composed mainly of sodium sulfate. Compared with sodium chloride, the sulfate is a very unsatisfactory curing agent, as McLaughlin and Theis[7] have shown.

Sheep skins are usually de-wooled in domestic packing plants immediately after slaughtering and flaying, and the curing process is thus obviated, the de-wooled skin being pickled with acid and salt before shipment to the tannery. A small proportion of the sheep skins produced is not de-wooled and is cured with sodium chloride, in much the same manner as in the case of cattle hides and calf skins. Most of the sheep skins which are imported are in the pickled state.

Principles of Curing

This subject is of great importance because any deterioration of a skin before or during curing is of irreversible nature; poorly cured skin will not produce the highest quality leather, regardless of the tanner's care or skill. Of equal importance is the fact that lack of uniformity in curing makes it doubly difficult to maintain uniformity of tannery processes and of the finished leather. And, on the other hand, no adequate understanding of tanning processes is possible without knowledge of curing.

Prior to 1921 no systematic investigation of the mechanism of curing had been made. In view of this, curing was the first problem studied by the Cincinnati Laboratory.[8,9] These investigations necessarily included both chemical and bacteriological studies. It was quickly realized that in view of the variable nature of the material being studied, and of the many factors involved, a great number of observations would have to be made if dependable average results were to be obtained. Prior to the publication of the principles evolved, it was possible to prove them by large scale tests. This was made possible through the cordial cooperation of a number of packers. Thus a large number of heavy hides were used in large scale curing experimentation, and accurate records were kept of the finished leather results.

Bacteriology of Curing

As has been noted, animal skin is covered with bacteria. Since the isolation and identification of all these many bacteria would have been an endless and probably useless task, McLaughlin and Rockwell[8] decided to study only enough of them to establish their great variety. Consequently, thirty separate heavy steer hides, free of manure, were sampled as they dropped from the carcass. Part of each sample was soaked in sterile distilled water and another part in sterile nutrient broth media, and all specimens were incubated at temperatures ranging from 12 to 56° C until putrefaction occurred. The resulting growths were then examined, both microscopically and culturally, and their morphological and biological characteristics were determined. Microscopic examination revealed that a wide variety of microörganisms were present on fresh hide, including: cocci, staphylococci, streptococci, both gram positive and negative; bacilli, large and small; motile

and non-motile, both gram positive and negative; spirilla and spirochaeta, together with yeasts, oidia, molds, and protozoa.

From the whole group of bacteria, twenty-four predominating strains were selected and studied in detail. These examinations included: size and shape, gram stain method reaction, spore formation, motility, nature of colony growth on agar and on potato, milk, and broth, nitrate production, indol production, digestion of gelatin, coagulated serum, coagulated egg, as well as digestion of the specially prepared hide fractions, collagen, mucoid, albumin and globulin, and elastin. Fermentation studies were made with seventeen different sugars, together with temperature and respiration requirements. Growth in calcium hydroxide-containing media was determined, and none were found to grow in media containing lime equivalent to a saturated aqueous lime solution. This elaborate identification system was found to be necessary in order to be certain that definite and distinct strains were being studied.

As a result of the examinations noted above, a great variety of form and characteristics of skin bacteria was found. Some were able to digest protein and some could not; all strains were either aerobic or facultative, and they varied in their ability to ferment carbohydrates. It was therefore decided to designate the bacterial phenomena to be studied as resulting from the action of two general groups of bacteria: the proteolytic and the non-proteolytic. The growth of proteolytic bacteria is encouraged by available protein substances, the non-proteolytic by carbohydrates. We shall now discuss the most important factors in the digestion of skin by bacteria.

Effect of Type of Bacteria upon Corium

As we have seen in Chapter 2, the derma or corium is the leather-forming material of skin. This being true, it is important to establish the action of bacteria upon the corium. Heavy hide corium was obtained by mechanically splitting off the thermostat layer and flesh of a steer hide. Not more than one hour elapsed between the death of the animal and the inoculation of its corium, as about to be described. The corium employed was not sterilized, but it was clean, in the sense that it contained no surface blood, filth, or fat. The bacteria used for inoculation were isolated from a broth solution in which fresh whole hide had been allowed to decompose.

Three 25-gram specimens of the corium prepared as above were treated as follows:

One specimen was smeared on both sides with a mixture of proteolytic bacteria, the second specimen with non-proteolytic bacteria, and the third specimen was not inoculated. The three specimens were immediately placed in separate containers with 250 ml sterile distilled water and were incubated for 72 hours at 20° C. At the end of this period the specimens were removed, weighed, and examined. The control showed slight decomposition and had

gained 1.4 per cent in weight; that inoculated with proteolytic bacteria showed much depletion and decomposition and had lost 0.1 per cent in weight, while the non-proteolytic inoculated specimen showed no decomposition and had gained 4.4 per cent in weight. At the end of the water soak described, each specimen was placed for 24 hours in a saturated calcium hydroxide solution at 20° C to determine what effect, if any, the bacterial treatment had had upon the ability of the corium to swell in alkaline solution. At the end of the lime treatment the specimens were again carefully weighed, and their weight relation to the original uninoculated weight was calculated and showed: control, 5.9 per cent gain; proteolytic, 3.3 per cent loss; and the non-proteolytic, 10.9 per cent gain.

The experiments described above were performed with unsterilized corium. They were repeated with corium which was sterilized by means of hydrogen peroxide prior to inoculation. At the end of the combined water soaking and liming treatment, the control had gained 3.8 per cent in weight, the proteolytic had lost 12.4 per cent, and the non-proteolytic had gained 12.5 per cent.

These experiments indicate that the skin corium is very sensitive to proteolytic bacterial action.

Effect of Bacterial Food

Four 20-gram clean (unsterilized and uninoculated) fresh corium specimens were treated as follows:

One specimen was placed in sterile distilled water; a few drops of whole beef blood were smeared on both sides of the second specimen, and it was then placed in water; the third specimen was placed directly into a three per cent dextrose solution; and the fourth specimen was smeared on both sides with blood and then placed in a three per cent dextrose solution. At the end of 48 hours incubation at 20° C all specimens were removed and placed for 24 hours in a saturated calcium hydroxide solution, after which they were removed, then weighed and examined. The first, or control, specimen showed 12.2 per cent weight gain; the second, 1.7 per cent loss; the third, 13.4 per cent gain; and the fourth, 19.1 per cent gain. Part of the weight gain noted with dextrose is no doubt due to the swelling effect of its fermentation products.

Effect of Temperature

The growth and activity of skin bacteria are greatly affected by temperature, as would be expected. This subject will be dealt with in detail in connection with the effect of salt in curing.

Effect of Gaseous Environment

As noted above, all the isolated skin bacteria which were found to be proteolytic were either aerobic or facultative, that is, they were able to grow

under both aerobic and anaerobic conditions. But their ability to digest protein is greatly inhibited under strictly anaerobic environment. This was determined by inoculating tubes of coagulated blood serum with typical skin bacteria and then incubating the tubes for 24 hours at 37° C under varying gaseous environment; the results were as follows:

There was great digestion under ordinary aerobic conditions or when oxygen was introduced and very slight digestion when the environment was made anaerobic. These experiments were extended whereby pieces of uncleaned fresh whole hide were placed in distilled water and incubated for 24 hours at 25° C under various conditions, and at the end of the soaking period the various soak waters were examined for proteolytic bacterial growth. Where plain water was employed, a fair amount of growth resulted; when oxygen was bubbled through the water, there was large growth; when carbon dioxide was bubbled through the water, a small growth resulted; when the water contained three per cent dextrose and carbon dioxide was bubbled through, there was no growth; but if the carbon dioxide was replaced by oxygen, a fair amount of growth occurred.

Reaction of Environment

It is well known that the behavior of enzymes is greatly influenced by the reaction of their environment. The growth of skin bacteria and their proteolytic power are also greatly influenced by environmental reaction. A piece of fresh whole steer hide, 2.5 × 4.0 inches, was placed in 300 ml distilled water which was maintained at a pH value of 8.0 by additions of calcium hydroxide and was incubated for 72 hours at 25° C. A duplicate specimen was maintained at pH 5.0 by means of acetic acid. At the end of the incubation period the hide soaked in the alkaline solution was greatly decomposed, and the solution contained many proteolytic bacteria. The specimen soaked in the acidic solution showed no decomposition, and its solution contained only a very few proteolytic bacteria but many non-proteolytic.

Having thus noted the general characteristics of skin bacteria, we will now consider their control—in other words, the effect of each important factor upon the antiseptic value of sodium chloride, as determined by McLaughlin and Rockwell.[8] These factors include: concentration of sodium chloride; presence of nutritive substances such as blood, dissolved skin protein, or manure in the curing brine; the effect of temperature; and the reaction of the brine and effect of the respiratory conditions.

When in the following experiments a mixed culture of bacteria is stated, this refers to the growth of mixed bacteria which resulted from inoculation of broth with fresh hide for 48 hours at 20° C. In estimating the number of bacteria, the dilution plate method was employed. This does not, of course, take into account the obligatory anaerobes but this is not important for,

as pointed out above, the great majority of skin bacteria are aerobic or facultative. However, in order to eliminate any large source of error, the estimations were controlled by examination of stained and fresh preparations from each experiment. All sodium chloride or blood percentages given are the final concentrations in the solution based upon volume.

Antiseptic Value of Sodium Chloride

Table 29 illustrates both the antiseptic power of sodium chloride toward skin bacteria and the modifying of this power by the addition of blood serum. One part by weight of whole fresh steer hide was incubated for 72 hours at 20° C in five parts of broth to which had been added the percentages of sodium chloride shown. Growth and lack of bacterial growth are indicated by plus and minus signs respectively.

Table 29.

	% NaCl								
	2	6	10	12	14	16	18	22	26
No Blood Serum Added	+	+	+	+	+	+	–	–	–
5% Blood Serum Added	+	+	+	+	+	+	+	–	–
10% Blood Serum Added	+	+	+	+	+	+	+	+	–
30% Blood Serum Added	+	+	+	+	+	+	+	+	–

We note that whereas growth was restrained by 18 per cent of salt, 22 per cent was required in the presence of five per cent of blood serum. The effect of whole beef blood on the antiseptic power of sodium chloride is shown in Table 30, where one part fresh whole steer hide was placed in four parts sodium chloride solution of the concentrations shown and incubated for 24 hours at 22° C. The figures shown are the number of bacteria present in 1.0 ml of brine.

Table 30.

Per Cent NaCl In Brine	No Blood	10 Per Cent Blood In Brine
5	67,000,000	424,000,000
10	34,000,000	47,000,000
15	1,480,000	18,640,000
20	1,010,000	18,840,000
25	1,170,000	15,520,000
30	630,000	2,380,000

Analysis of the brine running from packs of steer hides during curing in packing houses showed the brine to contain 40 per cent of blood at the end of the first hour of curing and eight per cent at the end of four weeks.

Effect of Temperature

Fresh steer hide was placed in broth to which sodium chloride had been added and was incubated at 20° C for nine days. The results are given in Table 31, where the numbers shown are the bacteria per 1.0 ml.

Table 31.

Temperature (°C)	4 Per Cent NaCl Broth	10 Per Cent NaCl Broth
37	1,100,000,000	47,000,000
20	879,000,000	4,520,000
10	218,000,000	2,470,000
5	10,820,000	51,100

Rate of Bacterial Growth

The rate of bacterial growth is determined, among other factors, by temperature and available food. This is shown in Table 32, where the growth of a mixed culture from fresh steer hide is expressed as number of bacteria per ml.

Table 32.

Hours of Incubation	Broth at 37° C	Broth at 20° C	10 Per Cent NaCl Broth at 20° C
0	10,100	11,800	10,600
1	16,900	13,300	11,200
2	28,600	17,700	11,500
3	300,000	25,600	11,300
5	9,490,000	71,000	10,800
24	206,000,000	356,000,000	60,100

In considering this table in connection with the curing process, it will be recalled that salting is not begun until some two hours after the death of the animal, and also that the surfaces of the hide contain much blood and other organic filth.

Effect of Reaction

Table 33 shows the effect on the growth of a mixed culture of hide bacteria in broth when the pH value of the broth is varied by the addition of hydrochloric acid. Growth or lack of growth is indicated by a plus or minus sign respectively.

Table 33.

pH Value of Plain Broth	6.69	6.26	5.00	4.74	4.48
Growth	+	+	+	+	−
pH Value of Broth + 10% NaCl	6 70	6.20	5.00	4 30	
Growth	+	+	−	-	

Table 33 shows that whereas the bacteria grew in plain broth at a pH value of 4.74, growth ceased at pH 5.00 in the presence of 10 per cent sodium chloride.

Having considered the important bacteriological factors in curing, we shall now turn to the more strictly chemical aspects, although it must be kept in mind that the chemical and bacterial phenomena are closely interrelated.

Chemistry of Curing

In approaching the chemical phases of curing, McLaughlin and Theis[9] considered that the important problem must be to impregnate the hide or skin

with salt as rapidly as possible after the body heat had escaped from the flayed hide.

When a hide or skin is "salted," it is simply covered with salt on both surfaces, as described above. When it is "brined," as in the case of Argentina Frigorificos, it is placed directly, after washing, into a saturated salt solution. The curing salt must be dissolved in water before it can penetrate the hide. This means, therefore, that in the case of salting, the curing process will not commence until the salt crystals have extracted water from the hide and have thus formed a brine. In the brining process, however, all of the bacteria present on the hide are immediately subjected to the antiseptic action of the preformed salt solution. With these thoughts in mind, McLaughlin and Theis performed the following experiments.

Water Content of Fresh Hide. Heavy, short hair steer hide was found to contain an average of 61.66 per cent water. The water present in the hide was not uniformly contained throughout its thickness: the upper 20 per cent of the hide excluding the hair—in other words, the thermostat layer—contains 74.35 per cent water, the middle 50 per cent of the hide 61 per cent, and the lower 30 per cent (including the flesh) 54.10 per cent.

Movement of Salt into Hide. When a hide is salted immediately after flaying, the salt is found to have diffused into the hide during the early hours of curing mainly from the flesh side; comparatively little having diffused through the hair or grain surface, as shown in Table 34, where the NaCl percentages shown are based upon the weight of the fresh hide, with the weight of the hair excluded.

Table 34.

Per Cent NaCl Absorbed								
1 Hour Salting Hair Side	4½ Hours Salting Hair Side	24 Hours Salting Hair Side	1 Hour Salting Flesh Side	4½ Hours Salting Flesh Side	24 Hours Salting Flesh Side	1 Hour Salting Both Sides	4½ Hours Salting Both Sides	24 Hours Salting Both Sides
0.00	0 00	0 23	1.20	2 97	5.70	1.05	2.86	5.45

Factors which Prevent Rapid Movement of Salt into Hide. A number of conditions retard the diffusion of salt into the hide. One of these is the lapse of time between flaying and salting. This is shown in Table 35, where the hide was salted on both sides after varying periods of delay, and the amount of absorbed salt was determined at the end of one hour of salting or of brining in a 25 per cent NaCl solution.

Table 35.

	Per Cent NaCl Absorbed After Delay in Salting of				
	None	1 Hour	2 Hours	4½ Hours	6 Hours
Salting	1.65	1.14	1 04	0.39	0.42
Brining	2.64	1 96	1.79	—	1.19

The striking results shown in Table 35 obtained whether the hide specimens were or were not exposed to the atmosphere during the periods of delay, showing that the decreased salt absorption was not due to surface evaporation.

Table 35 shows that the bulk of the salt which a hide absorbs *during the early hours of curing* enters through the flesh side. When the hide is flayed, its flesh side is invariably covered with blood. This blood delays salt diffusion into the hide, as the following experiments show:

Fresh hide was divided into two specimens which were salted on both sides as in regular practice, but one of the specimens was freed of the blood on the flesh side before salting. The two specimens were then salted for varying time periods, and salt absorption was determined; the results are shown in Table 36.

Table 36.

Time of Salting	Per Cent NaCl Absorbed	
	With Blood	Blood Removed
1 hour	1 05	1.82
4½ hours	2.21	2.79
24 hours	6.36	6.51

We note a very much greater salt absorption during the early, and most important, hours of curing in the absence of blood. At the end of the 24-hour period the salt absorption is about equal. This is not true, however, when, in addition to the effect of flesh side blood, delayed salting treatment occurs as well, as shown in Table 37.

Table 37.

Hours Delayed	Time of Salting	Per Cent NaCl Absorbed	
		With Blood	Blood Removed
4½	1 hour	0.26	1.34
4½	24 hours	4.11	6.92

Tables 36 and 37 show also that even in the absence of blood, delaying the salting treatment for 4½ hours results in a decreased salt absorption during the first hour of salting.

In view of the apparently great importance of blood in curing, McLaughlin and Theis collected samples of the brine running from hide packs, and determined their blood content, with the results shown in Table 38.

Table 38.

Salting Period	Per Cent Blood in Brine
1st hour	42.6
3rd hour	21.4
4th hour	17.7
5th hour	14.2
1 day	9.7
4 weeks	8.0

In order to determine further the effect of blood, two additional experiments were run. Two specimens of fresh hide were placed in saturated sodium chloride solutions, one of which contained 20 per cent of its weight of whole fresh beef blood. At the end of two hours of such brining treatment

the specimens were removed and analyzed for absorbed salt, with the results shown in Table 39.

Table 39.

Temperature of Brine (°C)	Per Cent NaCl Absorbed No Blood	Per Cent NaCl Absorbed 20 Per Cent Blood
11	2.24	1.89
24	3.65	3.07
37	3.43	2.47

The other experiment was designed to simulate the action of hide blood in preventing salt diffusion. Paper extraction thimbles were immersed in fresh whole beef blood, the excess blood was poured away, and the thimbles were drained. The thimbles were allowed to stand for varying time periods, and the rate of salt diffusion through them was then measured as follows: The thimble was suspended in a beaker of distilled water, and 25 per cent sodium chloride solution was poured into the suspended thimble. At the end of one hour the amount of salt which had diffused through the coagulated blood membrane into the outside water was determined. The amount of salt which diffused through the unaged blood membrane control is taken as 100. The salt diffusion values when delayed treatments were employed are calculated in their relation to the control. The ratios of diffusion are given in Table 40.

Table 40.

Treatment	Ratio of Diffusion
Immediate	100
½ hour's standing before treatment	100
1 hour's standing before treatment	75
2 hours' standing before treatment	74
3 hours' standing before treatment	42
4 hours' standing before treatment	40
24 hours' standing before treatment	85

In view of the data given in this section and of general practical experience, there can be no doubt of the great effect of blood in curing.

Since the bulk of the salt taken up by the hide in the early hours of curing enters through its flesh side, the removal of adhering flesh and fat before salting is of great importance. Stuart and Frey[16] have shown that when adipose tissue is not removed, the entry of salt into the hide is greatly impeded.

Post Mortem Effects upon Swelling and upon Volatile Nitrogen Formation

In 1921, McLaughlin[10] showed that if fresh steer hide was allowed to lie in air at 21° C for varying time periods—without any curing treatment—the capacity of its corium to swell in saturated calcium hydroxide solution (containing excess undissolved lime) changed as a function of post mortem time. This was determined by mechanically removing the thermostat layer and flesh and weighing and placing the resulting corium specimens in the lime

solution for 120 hours, and again weighing them at the end of this time. The results are shown in Table 41, where the swelling capacity of the corium when placed in the lime solution within one-half hour after flaying is taken as 100, and the other values are related thereto.

Table 41.

Hours of post mortem	Swelling when corium was exposed to atmosphere during post mortem	Swelling when corium was kept in closed jars during post mortem	Swelling when whole hide was exposed to atmosphere during post mortem and corium obtained as needed
0.5	100	100	100
1.5	86	88	92
2.5	76	88	78
3.5	77	82	73
4.5	80	83	76

The reasons for the decreased swelling capacity of the corium as noted in the table are not entirely clear. But long experience has taught the packer that post mortem effects on the value of the hide are very great, and that salting must not be delayed after the escape of body heat. There are no quantitative data available which correlate the decreased swelling values, noted in Table 41, and leather results. Scattered experiments have been made of putting fresh, uncured hides directly into tannery soaks. The results with such fresh hides have usually been diappointing as to the weight yields and plumpness of the resulting leather, compared with properly cured hides. But in such experiments plain water was used in soaking; and we now know that such treatment was inadequate for the very necessary partial removal of coagulable proteins of the hide, which are soluble in the salt brine of curing and in the resulting salt soak solution of the tannery. Stather and Sluyter[14] have experimented with small hide pieces cured by both salting and by brining after 5 and 24 hours post mortem at 18° C, respectively, and found negligible differences in soaked, limed, and tanned weight gains. Their experiments did not include post mortem periods of less than five hours, so that no basis of comparison with the results of Table 41 is available. Packers have found, however, that a post mortem period of 24 hours is very undesirable from the standpoint of hide quality. As noted above, when unflayed calf carcasses are stored for 24 hours before flaying, the packer is careful to maintain a temperature not exceeding 1° to 2° C.

In 1930, Theis[18] studied post mortem effects in uncured skin and concluded that the initial stage of decomposition is enzymatic in character, and that the advanced stage is a combination of the action of enzymes of both bacterial and non-bacterial origin. He showed that only a negligible amount of ammonia was formed by skin decomposition during 24 hours post mortem at from 5° to 20° C. This finding has recently been confirmed by Koppenhoefer and Somer,[5] who show in addition that the increase of free fatty acid

content of hide corium is negligible during the same period. Somer[13] has suggested that the quality of curing may be determined by the amount of volatile nitrogen the hide contains. He gives interesting data of tannery leather yields plotted against volatile nitrogen content of the various hide lots, and the correlation between the two values is quite striking.

Dehydration and Salt Absorption in Curing

The rate of dehydration and of salt absorption by fresh heavy hide was determined during the first 24 hours of curing by salting, and by brining in a 25 per cent sodium chloride solution, at 20° C. The results are given in Table 42.

Table 42.

	Salting			Brining		
Hours Treatment	Shrinkage (%)	Dehydration (%)	NaCl Absorbed (%)	Shrinkage (%)	Dehydration (%)	NaCl Absorbed (%)
1	− 5.37	− 6.91	+ 1 54	− 5.76	− 8.15	+ 2.39
3	− 10.45	− 13.81	+ 3.36	− 9.63	− 13.12	+ 3.49
4	− 12.79	− 16.68	+ 3.89	− 10.90	− 15.06	+ 4.16
5	− 16.45	− 20.76	+ 4.31	− 11.81	− 16.48	+ 4.67
24	− 23.85	− 30.51	+ 6.66	− 13.45	− 21.21	+ 7.76

It will be noted that salt absorption during the important first 24 hours of curing is considerably greater in the case of brining. This is of significance, since it is the early hours of curing which are of importance in arresting bacterial decomposition of the hide. It was found, however, that when the brined hide was removed from the brine solution, drained, and then salted down in pack for four weeks, as in practice, that the shrinkage and salt content of the hides were similar whether they were salted or first brined and then salted. In either curing method the salt absorption follows a very definite course as a function of time of curing, at least up to 50 hours. This absorption follows the general equation: $Y = \frac{x}{a + bx}$, where Y = salt absorption, x = hours of curing treatment, and a and b are empirically calculated constants.

Summarizing the various investigations described above, we have learned that animal skin is covered with many bacteria which may attack and decompose it. The extent of such decomposition is governed by a number of factors and by the character of the curing method employed. The results all suggested the desirability of treating the hide or skin with a saturated sodium chloride solution rather than by salting with dry salt.

In addition to the better curing obtained by brining, McLaughlin and Theis pointed out that brining removes more of the coagulable proteins in the skin than does salting. This has been confirmed by Stather and Herfeld,[15] and more recently by Roddy.[12]

McLaughlin, Theis, and Rockwell realized the great variability of material such as animal skin, since many laboratory determinations of each phenome-

non were necessary in order to secure dependable average results. They realized, also, that isolated practical scale tannery experiments, entailing only a few hides or skins, were probably of but little value. In view of all this, they were enabled by several large packers to run conclusive tests of curing by regular salting versus curing by first washing the hide and then brining followed by salting. Some 2,000 heavy, short hair steer hides were involved in the first experiment. The hides were split down the backbone into sides as they reached the cellar floor, and right and left sides were alternated between the two curing methods. Thus, 2,000 sides were salted in the regular manner, and a corresponding 2,000 were washed, brined, and salted. Accurate blood or green weights were taken, together with the cured or shipping weights, as well as the tannery received weights. The 4,000 sides were shipped to a representative sole leather tannery, and the two types of cure were processed at the same time. Accurate weights of the resulting leathers were kept so that leather yields might be calculated both on "pure leather" weights and on finished sole leather. Pure leather weight refers to leather which was removed from the last layer, rinsed in water, and dried; such weight values eliminate yield variables due to subsequent finishing operations. Part of each cure was processed by a so-called "mellow" beam-house treatment, that is, long soak and mellow limes. The other part received a "sharp" treatment, involving shorter soak and sharper limes. The reason for giving the two different beam-house treatments was to demonstrate that the cleaner brined stock (from which surface blood and filth and much of its coagulable protein had been removed) required a shorter and sharper beam-house treatment. Previous experiments had shown that the beam house processes normally given regular salted stock tended to deplete the brined stock, which, as all tanners now know, responds to soaking and liming much more rapidly than does the regular salted. The results on the entire experiment showed as follows:

Per Cent Shrinkage from Blood to Tannery Received Weight.

Brined	19.00	Salted	17.86

White Weight Gain from Blood Weight

Brined stock made 0.5 point less than salted with mellow treatment, but 3.8 points more with sharp treatment, that is, from 120.0 to 123.8 per cent.

Pure Leather Yield on Blood Weight

The brined stock made 1.1 per cent more leather with the mellow treatment than did the salted and 1.7 per cent more with the sharp beam-house treatment.

Finished Leather Yield on Blood Weight

The brined stock made 0.2 per cent more leather with the mellow treatment than did the salted and 1.4 per cent more with the sharp beam-house treatment.

The second large-scale experiment involved some 20,000 heavy, short-hair steer hides. All the procedures of the first experiment were followed,

except that the hides were not split into sides before curing; that is, whole hides were alternated between the two curing methods. The weight gains and leather yields showed essentially the same comparison as in the first experiment. The brined hides of the second experiment, like the first, showed practically no salt stains, and the leather was again plumper than that from the regular cured hides.

The differences in weight yields noted above were small but appreciable; the leather from the brined stock was plumper and showed practically no salt stains. Of probably greatest importance was the uniformity of quality and condition shown by brined hides. DeBeukelaer[1] has recently reported large-scale experiments on brined hides as follows: they show higher white weight gains than the salted, equal or slightly superior leather yields, plumper leather, and freedom from salt stains. The same author has studied the brining of calf skins, and one of us has had opportunity to watch their behavior in the tannery. When properly processed, they produce superior upper leather which is singularly free from salt stains.

The early Cincinnati studies described above have been followed by many important investigations in various laboratories.

Kaye[4] obtained ox hide within 15 minutes after flaying, washed it, and then brined specimens in sodium chloride solutions of various strengths: 10, 15, 20, 23, 27, and 33 per cent; other specimens were dry-salted. Duplicate specimens were allowed to stand in room atmosphere, at 20-22° C, and samples were taken after 5, 11, 32, and 52 hours, and for 8 days and were then treated with the same salt solutions and with dry salt. The brined specimens were removed from the brine and were salted with dry salt for 1, 2, and 8 days, and for 3 months. All of the various specimens were examined microscopically at the end of the various curing treatments. Kaye's findings may be summarized as follows:

Hide must be cured within a few hours after death if it is to retain a condition approaching fresh hide. A hide salted immediately after death is fresher after several months storage than if allowed to lie around for several hours after flaying. Brining prior to salting was found to be advantageous, but the brine solution should be maintained saturated with salt, and its temperature should not exceed 22° C.

Bergmann[2] has made extensive studies of the curing problem and has concluded as follows:

"The hide should be washed immediately and carefully to remove all substances containing many bacteria and having a tendency to develop bacterial fermentation, *i.e.*, substances such as blood, dung, lymph, and other impurities. The next step is to provide the entire hide as quickly as possible with a high salt content, which can best be done by brining."

And in 1932 Bergmann[2] stated: "Well brined hides do not heat so readily as unbrined and soak back much better."

In summarizing their extensive curing studies, Koppenhoefer and Somer[5] state, among other conclusions: "The advantages of brining of freshly flayed hides can be reiterated on the basis of the results obtained by analyzing brine cured hides."

Roddy[12] has reported that brining prior to salting increases leather yields without harming any of the physical properties of the leather.

In 1922, McLaughlin and Theis[9] suggested, on the basis of numerous experiments, that the reaction of curing salt had an important bearing upon the speed of its diffusion into hide and its dehydrating ability, as well as in its antiseptic power. Curing salts vary greatly in composition and reaction. This has been shown by Bowker and Beck,[3] whose extended analyses we produce as Table 43. This table illustrates the very great variety of salt composition, and it shows that most curing salts are of an alkaline reaction.

Lloyd, Marriott, and Robertson[6] have studied the curing properties of salts of various reactions and have concluded that acid-reacting brine is best for preserving hides. And they show that in a 25 per cent brine solution the range of pH values at which hide bacteria can grow is between 6.0 and 8.0, confirming the original observation of McLaughlin and Rockwell. Stuart and Frey[17] have also investigated the effect of the pH value of curing salt upon bacterial growth by adjusting 25 per cent salt solutions to various pH values with H_2SO_4 and NaOH and then brining fresh calf skin in them. They found bacterial growth to be inhibited at pH 5.0, confirming the observations of McLaughlin and Rockwell and of Lloyd, Marriott, and Robertson.

If curing is accomplished with an acid-reacting salt or brine, mold growth may be encouraged. This may be prevented by the addition of the proper fungicide to the curing salt or brine.

Salt Stains

Brined hides or skins very rarely show a defect known as "salt stain." Salted stock often does, and the poorer the cure and the dirtier the hide or skin, the more frequent and numerous are the stains.

Salt stains in salted hides or skins vary in appearance from a light brown to a greenish blue color. They are difficult and often impossible to remove, and they lower the value of the finished leather. The cause of the stains has been investigated by numerous workers, and from their studies it has been established that salt stains are of several varieties. They may be produced by bacterial action or may result from the presence of certain iron compounds which are present in the skin, itself, or which may be found in the curing salt. The most successful preventive of salt stains in salted stock appears to be the addition of from 3 to 5 per cent of anhydrous sodium carbonate to the curing

Table 43. Analyses of Salts Used for Curing Skins

Sample No.	Source	Type	Commercial Designation	Moisture %	Insolubles %	Iron Fe %	Calcium Ca %	Magnesium Mg %	Sulphate SO_4 %	Carbonate CO_3 %	pH	Sodium Chloride NaCl %
1	New York, Watkins Glen	Evaporated	Vacuum Common Fine (Dried)	0.20	0.0	0.0001	0.42	0.01	0.67	0.01	7.3	98.4
2	New York, Watkins Glen	Evaporated	Coarse Common Fine (Dried)	0.53	0.4	0.002	0.38	0.06	0.53	0.01	8.2	97.7
3	New York, Watkins Glen	Evaporated	Coarse Common Fine (Undried)	0.53	0.2	0.0004	0.39	0.02	0.42	0.01	7.3	98.0
4	New York, Watkins Glen	Evaporated	Ground Alum	1.22	0.2	0.0002	0.49	0.08	0.51	0.02	8.0	96.8
5	New York, Retsof	Mined	Coarse "C"	0.02	0.84	0.0000	0.20	0.01	0.30	0.00	7.4	98.4
6	Michigan, Detroit	Mined	Coarse "C"	0.10	0.64	<0.0001	0.26	0.01	0.40	0.00	7.4	98.4
7	Louisiana	Mined	Coarse	0.01	0.2	0.0000	0.17	0.00	0.26	0.00	7.0	99.3
8	Louisiana	Mined	"C"	0.02	0.47	0.0000	0.11	0.01	0.20	0.00	7.1	99.1
9	Kansas	Mined	Kansas Crushed Rock Salt No 1	0.07	0.68	0.0000	0.14	0.01	0.24	0.00	7.2	98.8
10	Louisiana	Mined	"A" Grade	0.12	0.7	<0.0001	0.35	0.00	0.54	0.00	7.5	98.1
11	Michigan	Mined	Detroit "CC" Rock	0.02	0.5	0.0000	0.22	0.01	0.30	0.00	7.0	98.8
12	Kansas	Mined	No 7 Rock	0.04	1.6	0.0001	0.43	0.00	0.67	0.00	7.1	97.1
13	Michigan	Evaporated	Medium Evaporated	0.17	0.3	0.0003	0.22	0.00	0.22	0.05	7.4	98.8
14	Michigan	Evaporated	No 1 Granulated	2.51	0.5	0.0001	0.37	0.00	0.54	0.01	7.2	95.9
15	England	Evaporated	Liverpool Ground	0.24	0.1	0.0004	0.28	0.01	0.48	0.00	7.7	98.7
16	Kansas	Mined	No 7 Crushed Rock	0.03	1.7	0.0003	0.41	0.00	0.74	0.00	7.1	96.9
17	Ohio	Evaporated	Ohio River Salt No 1 Medium	1.39	0.1	0.0004	0.55	0.24	0.00	0.01	7.0	96.1
18	Ohio	Evaporated	Ohio River Salt No. 2	1.82	0.1	<0.0001	2.01	1.00	0.00	0.00	7.0	88.5
19	Ohio	Evaporated	Ohio River Salt No 1	1.97	0.1	0.0000	0.80	0.35	0.00	0.00	7.0	94.4
20	Ohio	Evaporated	Ohio River Salt No 2	1.69	0.1	0.0000	0.70	0.28	0.00	0.00	6.9	95.4
21	Michigan, St Clair Co	Evaporated	No 1 Granulated Evaporated Salt	0.19	0.0	0.0003	0.33	0.00	0.53	0.02	7.1	98.7
22	Michigan, St Clair Co	Evaporated	No. 1 Medium Evaporated Salt	0.19	0.1	0.0004	0.30	0.00	0.41	0.00	7.1	98.8
23	Louisiana	Mined	Rock Salt, Coarse	0.03	0.2	0.0000	0.09	0.01	0.12	0.00	7.1	99.5
24	New York, Halite	Mined	Rock Salt C	0.02	0.9	0.0000	0.18	0.00	0.29	0.00	7.3	98.5
25	Kansas	Mined	Crushed Rock Salt No 7	0.08	1.9	0.0002	0.25	0.01	0.47	0.00	7.3	97.2
26	Ohio, Cleveland	Evaporated	No. 1 V P Granulated	0.22	0.1	<0.0001	0.35	0.01	0.51	0.00	7.3	98.5
27	Ohio, Cleveland	Evaporated	No 1 Medium	0.58	0.1	0.0004	0.43	0.02	0.39	0.01	7.5	98.0
28	Ohio, Cleveland	Evaporated	No 1 Special G A	0.46	0.1	0.0009	0.37	0.01	0.37	0.03	7.9	98.3
29	New York (Western)	Evaporated	Ground Alum	0.45	0.1	0.0002	0.42	0.01	0.43	0.00	7.3	98.2
30	New York (Western)	Mined	No 1	0.02	1.2	<0.0001	0.33	0.00	0.44	0.00	7.6	97.7
31	Germany	Mined	Fine Calf	0.65	1.2	0.0000	0.12	0.07	0.35	2.63	8.2	93.1
32	Germany	Mined	Coarse	0.10	0.6	0.0000	0.09	0.11	0.33	0.01	7.1	98.6
33	Germany	Evaporated	Commercial Denatured	0.52	1.4	<0.0001	0.48	0.10	0.72	0.02	6.9	96.2
34	England	Evaporated	Salt	0.29	0.1	0.0001	0.32	0.00	0.77	0.03	7.7	98.2
35	England	Evaporated	Broad Salt	0.45	0.1	0.0001	0.20	0.01	0.71	0.02	7.5	98.3

NOTE Value for moisture, insolubles and sodium chloride were calculated as percentage of the samples as received, all other constituents as percentage of the dry soluble matter

salt. This method is due to Paessler,[11] who ascribed the stains to bacterial growth; the addition of the sodium carbonate is presumed to raise the alkalinity of the skin to a pH value at which bacteria cannot grow.

As stated above, however, when hides and skins are washed and brined before salting, the *causes* of salt stains are automatically removed.

Red Heat

Under this general term is included a variety of coloration which often appears upon the flesh side of salted hides and skins. And it may also appear on stock which has been brined with *alkaline* reacting salt solutions. The coloration is usually of a reddish or pink caste, but it is sometimes of a purplish shade. The stains do not, as a general rule, damage the skin or the resulting leather, since most of the chromogenic organisms causing the stains are evidently not proteolytic. The subject has been especially investigated by D. J. Lloyd[6] and her colleagues, by Bergmann,[2] and by Stuart and Frey.[17]

Lloyd, Marriott, and Robertson state that the red heat coloration is produced by halophilic organisms. These chromogenic bacteria are not usually found on fresh hides; they are added to the hide through the use of certain marine salts. The growth rate of the organisms is much slower than that of ordinary hide bacteria. Growth may be prevented by rendering the curing brine acid in reaction, through the addition of an acid salt. Two types of chromogenic *cocci*, one of which is a *red sarcina* and the other a *yellow sarcina*, were isolated from stained hides. The red variety appeared to be non-proteolytic, whereas the yellow variety was proteolytic. Bergmann[2] has also concluded that the colorations referred to are caused by chromogenic, halophilic bacteria, some of which can grow in an alkaline environment which is of a pH value as high as 9.0. He isolated the following chromogenic organisms from the stained areas of cured hides: *sarcina lutea, sarcina aurantica, micrococcus roseus, micrococcus tetragenus, proteus variety, actinomyces variety,* and *bacillus subtilis*. All these bacteria were capable of liquefying gelatin, and two were fat-cleaving. Stuart and Frey[17] found that chromogenic, hide-reddening bacteria were unable to grow in a medium whose pH value was 5.0; growth occurred at pH 5.5 only after six weeks of incubation at 30° C. But they pointed out that such pH values may encourage mold growth. They made an extensive study of the most efficient fungicides to be added to the salt, and the interested reader is referred to this important article.

Lack of space has prevented our giving more than a brief outline of the important subject of curing. But we have endeavored to outline the important principles underlying curing, and their practical application. Since both the principles and their application are simple, it is difficult to understand why so little progress has been made throughout the world in the curing

of hides and skins during the past two decades. Possibly the conditions of keen competition which lie ahead of the leather industry will stimulate it to action in this profitable and most important field.

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Chapter 8

Soaking

The first tannery process given the cured hide or skin is termed "soaking"; that is, it is soaked in water or in an aqueous solution of a chemical. This process is of great importance, for if it is improperly performed, the final leather will usually be defective. The soaking process must be understood both in itself and in its relation to, and bearing upon, the subsequent processes through which the skin is to pass. The extent and kind of soaking process required vary with the nature and condition of the skin itself, with the subsequent processes, and with the kind of leather to be made. Since these factors vary from time to time and from place to place, no hard and fast soaking rules can be formulated. But we shall describe the objects of soaking, the principles which underlie their attainment, and finally the application of the principles which have been derived.

The salt-cured or dried skin reaching the tannery contains less than its physiologic water content; it contains proteins which were originally in a liquid state but are now partially dried and often coagulated; the salt-cured skin contains salt; and its outside surfaces are covered with more or less extraneous matter and filth, together with many bacteria. The objects of soaking are: to rehydrate the skin proteins; to partially solubilize and remove the denatured dissolved proteins; to open up the contracted fibrous structure of the skin; to remove the curing salt in the case of salted skins, and to clean off surface filth. The cured skin contains many proteolytic bacteria. These bacteria are held in check by the presence of curing salt or, in the case of dry unsalted skin, by the absence of free water. When, in either case, the restraining influences are removed and when conditions become favorable for bacterial growth, bacterial digestion of the skin commences. It is necessary to prevent such digestion. The reader will therefore realize that soaking must be considered in both its chemical and bacteriological aspects, and that the two are interrelated.

The composition of animal skin is described in Chapter 3; but for our soaking studies, it will be here noted that skin is composed of various proteins, salts, and fatty bodies, in addition to water. The main protein constituent is collagen, together with smaller quantities of so-called coagulable proteins, such as albumin, globulin, mucoid, together with blood proteins.

Before 1923 no systematic investigation of the soaking process had been

published. McLaughlin, Theis, and Rockwell[3] therefore undertook to study it from both the chemical and bacteriological approach. We shall first describe their results and then refer to the many important subsequent studies of other workers.

Swelling of Cured Hide or Skin in Soaking

The extent to which cured skin substance can swell in water or in a saturated lime solution is a function, among other things, of the extent to which the hide or skin has dried in curing and before soaking and liming. The bend of a heavy salt-cured, short-hair steer hide contains approximately 41 per cent water and 12.50 per cent sodium chloride. Specimens of such a bend were dried to varying moisture contents by subjecting them to flowing air at 33° C. After drying, they were placed in distilled water at 20° C. They were removed at the end of 24-hour periods, surface moisture was removed by blotting, and they were then weighed. The specimens were then put back into fresh distilled water, and this treatment was continued until a maximum of swelling had been reached. Following the water-soaking treatment, each soaked specimen was placed in a saturated calcium hydroxide solution containing excess lime at 20° C and remained therein for 120 hours. They were then removed, blotted, and again weighed, after the now loosened hair had been mechanically removed. The final weight percentage after soaking and liming is termed the "white weight" in tanning parlance.

The results of these experiments are given in Figure 36, where all weight percentage results are based upon the weight of the original undried, cured hide, which contained 41 per cent water. The figure shows that whereas the control gained 30 per cent in weight at the end of 48 hours of water treatment, the specimens which were dried gained progressively less, as a function of the extent of drying. Thus, for example, the specimen which was dried to contain 29 per cent of water gained only 15 per cent over its original undried weight. We note also that the drier the hide, the longer is the soaking period required for maximum soak weight gain. The figure shows, too, that the percentage of white weight gain (that is, the relation between unsoaked and limed weight) is directly related to the extent to which the hide has dried.

Similar experiments were conducted with cured calf skin, with results essentially the same as those obtained with cured steer hide. From all these results we can appreciate, also, the importance of a uniform moisture content throughout the area of the hide or skin as it enters the tannery soak. If the skin has dried out in spots and such skin is given the normal soaking period, the dried spots will be undersoaked; conversely, if the soak is sufficiently extended to hydrate the previously dry spots, the remainder of the skin will have been oversoaked.

Since we shall frequently discuss the swelling of hide or skin in connection

with soaking and liming, it may be well to point out that the degree of swelling desired may vary with a process or a finished product. But the ability of the skin to attain a normal maximum swelling (even though such maximum may not be employed) is of great importance, and is one of the best gauges of the intrinsic value of the skin. The skin is usually completely hydrated at the

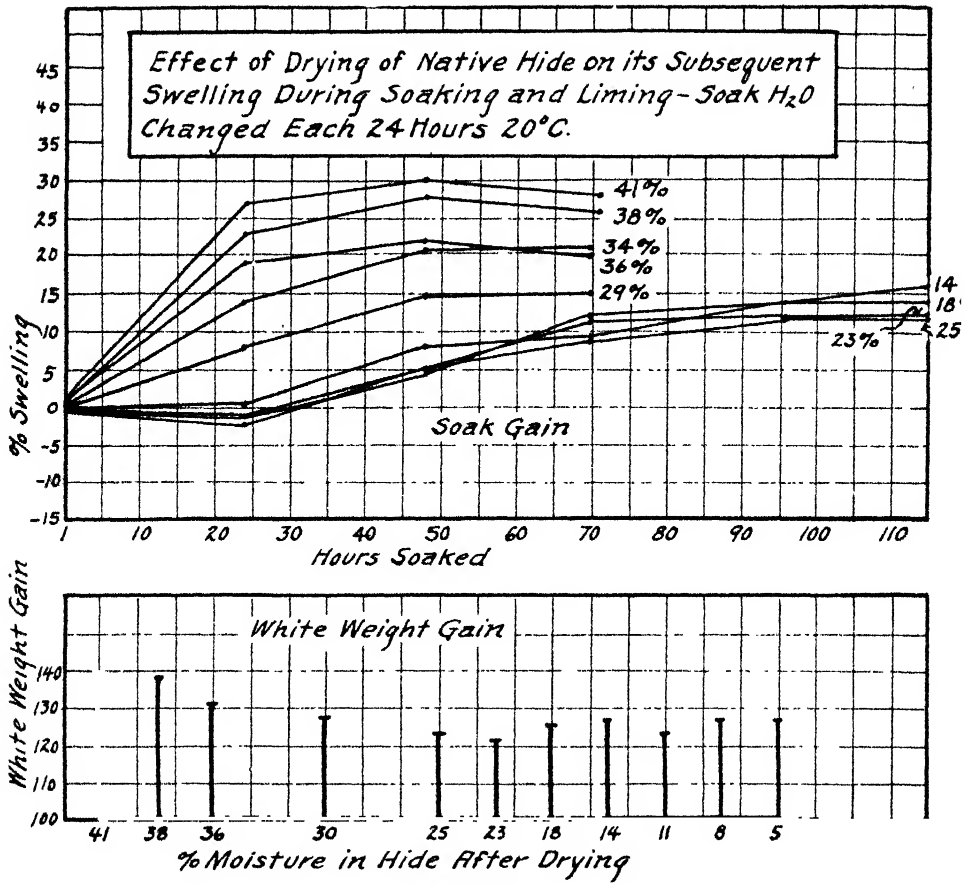

Figure 36

point of its maximum swelling in the soak. And if the soaking period is extended beyond this point, the skin loses in hydration and commences to dissolve. As a result of the investigations here described, many tanners have reduced their soaking period to conform with the point of maximum swell and have thereby obtained better results in leather yields and plumpness.

Character of Nitrogen Dissolved in Soaking

It was noted in Chapter 3 that animal skin contains in addition to collagen, its principal protein constituent, other proteins such as albumin, globulin, and mucoid. McLaughlin and Theis in 1923 pointed out that these were coagulable by heat or by acid and that their removal in soaking greatly affected the ability of the skin to swell. This is because these proteins become hardened during curing and tend to cement the collagen fibers together, thus preventing their separation and swelling. They termed them "coagulable" proteins, and divided the nitrogen which was found dissolved in soak water into the coagulable and uncoagulable. Since the coagulable proteins present in cured skin are more soluble in sodium chloride solutions than in plain water, numerous experiments were made in which both cured heavy hide and cured light skins were soaked in varying concentrations of sodium chloride. The data given in Table 44 are typical of these experiments. Table 44 shows the amount

Table 44

Initial NaCl Percentage of Soak	Final NaCl Percentage of Soak	Per Cent Total Nitrogen in Soak Solution	Per Cent Coagulable Nitrogen in Soak Solution
0.00	2.83	0.085	0.012
0.55	3.18	0.087	0.012
1.10	3.74	0.100	0.019
5.08	6.82	0.096	0.024
7.66	9.45	0.094	0.032
10.13	12.49	0.098	0.046
12.53	14.58	0.085	0.031
15.32	16.61	0.084	0.032
18.00	19.40	0.085	0.029
20.25	21.55	0.062	0.027

and kind of nitrogen in the soak water when one part of cured heavy steer hide was soaked for 24 hours at 20° C in four parts soak water or salt solution. Since the cured hide contained about 12.5 per cent of sodium chloride, and since this salt readily diffused into the surrounding soak water, the control experiment, it will be noted, showed a final soak salt concentration of 2.83 per cent, as would be expected. The difference in salt concentration between the first and second columns of the table in each experiment is explained by the outward diffusion of the curing salt.

It will be noted that the amount of coagulable nitrogen extracted from the hide increases with each increment of salt concentration up to 12.5 per cent, and that it decreases with further salt concentration. As will be seen later, there was practically no bacterial growth in the solutions containing the higher salt concentrations.

The closely knit epidermal layers of the skin do not permit the outward diffusion of the colloidally dispersed coagulable proteins which are removed

in soaking; these proteins diffuse out of the skin through its flesh side. This was found to be true by means of the following experiment:

Pieces of heavy steer hide were clamped into a flanged eight inch copper cylinder, so arranged that the soak water could freely penetrate the flesh side of the hide from below. Water was then poured into the cylinder and rested upon the hair or grain side of the hide. In this way, McLaughlin and Theis were able to separately analyze the soak water for the amount and nature of nitrogen which had come from the hair side and that which had diffused from the flesh side.

Employing this method, both domestic and Frigorifico hide were soaked for 48 hours at 20° C, and at the end of this period the soak water was analyzed. It was found that 60 per cent of the total nitrogen derived from domestic hide came from its flesh side and 40 per cent from the grain, and the corresponding figures for the Frigorifico hide were 56 and 44. When the domestic hide was well fleshed before soaking, the figures changed to 68 and 32 per cent. The important finding was that no coagulable protein was derived from the grain side in the three experiments. All of the coagulable nitrogen found in the soak was derived from the flesh side.

Effect of Temperature in Soaking

Salt-cured calf skin was soaked for two successive 24-hour periods at varying temperatures, in four parts water for each one part skin. The amount of total nitrogen dissolved, based upon the original skin weight, and the swelling of the skin are shown in Table 45.

Table 45

Temperature of Soak (° C),	% N Dissolved First 24 Hrs	% Swelling First 24 Hrs	% N Dissolved Second 24 Hrs	% Swelling Second 24 Hrs	% N Dissolved 48 Hrs	% Swelling after 48 Hrs
4	0.14	39.0	0.06	4.0	0.20	43.0
20	0.16	38.0	0.06	3.5	0.22	41.0
25	0.16	35.0	0.08	- 2.0	0.24	33.0
30	0.18	36.0	0.13	- 6.0	0.31	30.0
38	0.20	32.0	1.38	- 22.0	1.58	10.0

The figures in Table 45 show large decreases in swelling as temperature exceeds 20°, and these changes are coincident with increased solution of nitrogen. These changes are not merely the result of increased solution of the skin as a function of rising temperature; they are brought about largely by bacterial growth and digestion of the skin. This is shown in Table 46, where one part of cured calf skin was soaked for 12 hours in water at varying temperatures and the bacteria were counted at the end of the soak period.

Since bacterial growth in soaks is of such great importance, the lag period and generation time of calf skin bacteria were determined; these data are shown in Table 47. By "lag period" is meant, in this instance, the amount

Table 46

Temperature of Soak (° C)	Number of Bacteria per ml Soak Water
5	26,000
10	40,000
15	62,000
20	110,000
25	1,360,000
30	7,200,000
37	7,100,000

of time elapsing between the entry of the skin into the soak and the point when active bacterial growth occurs. And by "generation time" is described the number of hours required by the bacteria to reproduce themselves.

Table 47

Temperature of Soak (° C)	Lag Period (hours)	Generation Time (hours)
5	48.00	——
10	36.00	12.00
15	18.00	6.00
20	10.00	2.00
25	4.00	1.75
30	2.00	1.50
37	1.75	1.50

Rate of Bacterial Growth in Soaks

Examination of many specimens of domestic (salted) and Frigorifico (brined and then salted) heavy steer hides showed that the former contained an average of some 43,000 bacteria per each gram of hide, whereas the latter contained 13,000. The domestic cure contained about twice as much soluble surface nitrogen as the Frigorifico. The sodium chloride content of hides of both cures was similar, about 12.5 per cent. When samples of both cures were soaked in four times their weight of soak water at 20° C for varying time periods, bacterial growth occurred as shown in Table 48; this expresses the number of bacteria found per ml of the soak solution.

Table 48

Hours Soaked	Domestic Cure (Salted)	Frigorifico Cure (Brined and then salted)
2	2,750	400
4	3,800	660
8	8,770	1,520
12	19,420	4,600
16	150,400	12,200
20	1,144,000	395,000
24	5,264,000	2,212,000
48	278,900,000	126,100,000
72	931,700,000	378,500,000

From Table 48 it will be noted that a sharp increase in bacterial growth occurs after 16 hours soaking at 20° C in the case of both types of cure. We shall now consider other factors which, in addition to temperature, affect the rate of bacterial growth in soaking.

Bacterial growth in soaks is very sensitive to the reaction of the soak water, as would be expected in view of the data shown in Chapters 6 and 7. When cured steer hide is soaked in four times its weight of water at 20° C for 36 hours, and when the pH value of the soak water has been adjusted by the addition of hydrochloric acid or sodium hydroxide, the amount of growth varies with the final pH value of the soak water. Thus domestic hide soak water at pH 4.98 shows 4,200,000 bacteria per ml; at pH 6.60, 70,000,000 and at pH 8.20, 6,300,000. Frigorifico cured hide shows, at the same pH values, 40,000, 49,400,000 and 1,375,000, respectively.

Bacterial growth in soaks is greatly affected by the concentration of sodium chloride present in the soak. This is shown in Table 49, where one part of steer hide was soaked in four parts water containing varying concentrations of sodium chloride at 20° C. Bacterial growth is shown in the table as the number per ml soak solution.

Table 49

NaCl Added to Soak (%)	Domestic Cure 24-Hrs Soak	Domestic Cure 48-Hrs Soak	Frigorifico Cure 24-Hrs Soak	Frigorifico Cure 48-Hrs Soak
Control	5,264,000	278,900,000	2,212,000	126,100,000
1.0	1,754,000	86,454,000	210,000	108,700,000
2.0	789,000	33,468,000	36,000	--
4.0	157,900	8,367,000	12,900	250,000
6.0	52,600	578,000	7,300	140,000
8.0	51,000	204,000	7,000	25,000
10.0	40,000	160,000	3,600	8,000

Soak bacterial growth is also stimulated by the presence of available, soluble protein present in the soak. Thus a poorly cured hide or skin, or one containing blood or manure, will stimulate bacterial growth to a much greater extent than if it were well cured and clean.

Effect of Varying Proportion of Skin and Water

The number of pounds of water in which each one pound of skin is soaked must be considered. In the case of salt (sodium chloride) cured hides or skins the salt which diffuses out into the soak water assists in the removal of the coagulable proteins on the one hand and tends to restrain bacterial growth in the soak on the other. The greater the amount of soak water employed, the greater will be the dilution of the salt. This may result in a decreased extraction of coagulable protein material, an increased bacterial growth, and consequently the solution of valuable collagen. On the other hand, too

little soak water will prevent the rapid, uniform, and complete hydration of the skin; the skin will not be properly conditioned for the subsequent processes, and inferior products are obtained. Many large-scale practical experiments have indicated that approximately four pounds of soak water per each pound of salt-cured hide or calf skin gives the best results.

Soaking Dry Hides and Skins

The soaking of dry hides and skins presents a special problem to the tanner. Soaking such stock in plain water is unsatisfactory because of the great length of time required and the consequent danger of bacterial attack, together with the fact that but very slight dispersion of the dried coagulable proteins occurs. As a result of this, the collagen fibers are unable to swell and open properly. The addition of acids or alkalies, or of sodium sulfide, to the soak water to expedite the skin's hydration has not proved satisfactory. McLaughlin and Theis found that soaking sun-dried goat skins or dry hides in a sufficient concentration of sodium chloride for 24 hours and then transferring them to fresh water for another 24 hours resulted in satisfactory and uniform hydration and the dispersion of and a greatly increased removal of coagulable proteins. This method of soaking has been used advantageously by tanners of both dry goat skins and dry hides. Salt soaking is also being successfully employed by tanners of salt-cured hides.

As stated above, it is not feasible to formulate specific rules for all types of soaking, since conditions, processes, and desired leather qualities vary. But it is now generally recognized that soaking bears very directly upon the behavior of the hide or skin in the subsequent processes through which it must pass, and upon the quality and quantity of the final leather product. Now that the principles have been formulated, the tanner is in position to apply them to best meet his own conditions.

There have been other valuable studies of the soaking process. In 1924, Kaye and Lloyd[1] pointed out that when animal skin dries, the interfibrillary proteins form a non-swelling, insoluble, and non-elastic deposit around the collagen fibers, preventing their proper soaking and swelling in water. Lloyd and Pickard[2] were later granted both British and United States patents covering the addition of certain polyvalent salts, such as sodium citrate, ferrocyanide, or pyrophosphate, to soak waters for the dispersion of the coagulated proteins of dry hides and skins. Pleass[5] has made an extensive microscopic study of dry hides and dry goat skins which were soaked in aqueous solutions of many different dispersive chemicals She summarizes her findings as follows:

"An examination of the effect of the addition of various salts to a soak liquor on the fiber structure of dried hides (Bolivian) and skins (Patna goat) has been made, and it appears that the addition of sodium chloride, sodium

nitrate, or acid solutions of sodium bisulphite is very beneficial in the case of hides. For goat skins, however, sodium chloride is of considerable use, but high concentrations are required. Better results can be obtained, however, by using mixtures of sodium chloride and nitrate in conjunction with a small amount of an alkali such as sodium carbonate. Substances which disperse coagulated interfibrillary proteins (such as sodium citrate) appear to be of more use for dried goat skins than for dried hides. Sodium sulphide is not a good agent for soaking back either dried hides or skins."

Roddy[6] has recently made histologic studies of the location of the coagulable proteins present in fresh, cured, and soaked skins and hides. He finds these proteins are selectively stained by means of a 1 per cent aqueous solution of Gentian Violet. In this way he has demonstrated that the coagulable proteins are located mainly in the thermostat layer, much less being stainable in the corium. But in this connection it will be recalled that McLaughlin and Theis were able to extract very considerable quantities of coagulable proteins from the corium of fresh steer, cow, and calf skins. Roddy[6] also confirms the observations of previous workers (see Chapter 7) that brining before salting produces a better cure and superior leather results than are possible by ordinary salting.

Roddy and Hermoso[7] soaked heavy steer hide for 24 hours in various solutions and determined the amount of coagulable nitrogen extracted. If we express the coagulable nitrogen extracted by their plain water soak control (containing around 3 per cent sodium chloride) as 100 and relate thereto that extracted by the other solutions, the following figures are found: 1 per cent sodium citrate, 124; 10 per cent sodium chloride, 121; 1 per cent magnesium sulfate, 118; 1 per cent borax, 116; 1 per cent sodium carbonate, 113; 1 per cent boric acid, 89; 1 per cent dimethylamine, 76; and 0.1 per cent sodium polysulfide, 92. When the percentage of the total dissolved nitrogen which is represented by coagulable nitrogen is calculated, the following figures are found: 1 per cent sodium citrate, 44; 10 per cent sodium chloride, 37; 1 per cent magnesium sulfate, 50; 1 per cent borax, 40; 1 per cent sodium carbonate, 43; water, 34; 1 per cent boric acid, 32; 1 per cent dimethylamine, 22 and 0.1 per cent sodium polysulfide, 42.

Roddy and Hermoso have found that the addition of sodium polysulfide to the soak is very effective in accelerating the soaking of both salted and dry hides; for dry hides they recommend a polysulfide concentration of 0.3 per cent on soak solution basis, proportionately less polysulfide being employed for the salted.

Theis and McMillen[9] have followed the changes in the basic amino acids—arginine, histidine, and lysine—of salt-cured steer hide during the soaking process. They found the amount of these acids to decrease as a function of the time of soaking, and as a function of bacterial activity of the soak.

Theis and Neville[10] have shown by dilatometric means that the hydration capacity of skin proteins decreases as a function of bacterial activity in the soak.

The question of the addition of a bactericide to soak waters has been frequently discussed. This problem has been dealt with at length by Shuttleworth and Sebba,[8] who have investigated the value of many different disinfecting compounds. But this question has lost most of its significance in the light of modern knowledge and practice. In the case of reasonably well cured salted stock there is no good reason for soaking it to the point of bacterial damage. If it is poorly cured, the soak should greatly be reduced in time, in any event. And in the case of dry hides and skins, practice has shown that a long soak in plain water- even if a disinfectant is present—does not condition the stock nearly as well as if it is soaked in salt solution of sufficient strength to prevent bacterial growth on the one hand and to remove coagulable material on the other, or if the newer method of a sodium polysulfide soak is employed. These remarks do not of course apply when hides or skins must be disinfected against pathogenic organisms, such as anthrax or hoof and mouth disease. The incidence of anthrax in imported hides and skins is becoming progressively less, and to such an extent that the Bureau of Animal Industry has recently announced its intention of eliminating all disinfection requirements for anthrax at American tanneries. And as to hoof and mouth disease, O'Flaherty and Doherty[4] have only recently evolved a very simple means of disinfection. This method consists of soaking the suspected hides or skins in four parts of water per one part skin weight, at 20-25° C for 24 hours, the soak water to contain one part sodium hydrogen fluoride per 10,000 parts soak water, and the pH value of the soak solution to be so adjusted that it will be between 6.2 and 6.5 at the end of the 24-hour period. This method has now been adopted as official by the B.A.I. Its advantages are its simplicity, economy, and effectiveness. Large-scale tests have proved it to have no deleterious effect upon the leather made from either heavy hides or light skins.

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9. Theis, E R., and McMillen, E L, *Ibid*, **XXIII**, 226 (1928).
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Chapter 9

Liming and Depilation

As Wilson stated in Volume I of the second edition of this monograph: "the commonest method in use today for effecting the separation of the epidermal system from the true skin is also one of ancient origin and is known as liming, from the fact that saturated lime water is used." This statement is still essentially true and although many modifications have been made, the tanner still uses, in the main, a lime suspension for aiding the removal of the epidermal system.

In general, hides and skins, after having been properly soaked, are immersed in an unhairing solution, the principal constituents of which are hydrated lime [$Ca(OH)_2$] and water. In most cases, a "sharpener" is added to expedite the depilation action. The hides or skins are allowed to remain in contact with this suspension until the hair can be removed satisfactorily.

McLaughlin[24] has pointed out that there are at least four major objectives in the liming operation: (a) to act chemically upon the epidermal system, so that the skin may be properly unhaired; (b) partially to saponify the skin lipids; (c) to swell and hydrate the skin proteins satisfactorily; and (d) to condition the skin chemically and physically for subsequent operations. These four points will be adequately discussed under appropriate headings.

A great many different modifications of the unhairing process are used by the tanner. These might be listed as: (a) the straight lime liquor, in which only $Ca(OH)_2$ and water are used; (b) the lime and sodium sulfide or sulfhydrate liquor, in which a given percentage of sulfide or sulfhydrate are used in conjunction with lime; (c) the lime and arsenic sulfide liquor, in which arsenic trisulfide is added to the lime liquor; (d) the lime and amine liquor, in which dimethylamine is added to the lime suspension; (e) the lime and cyanide process, in which a given amount of sodium cyanide is dissolved in straight lime liquor; (f) the enzyme and sweating process, in which enzymes play an important role.

In the manufacture of sole and belting leather only a very small amount of sodium sulfide is used and the pit system is usually employed. In the fabrication of fine calf leathers, the tanner may use a small amount of sodium sulfide in addition to lime, but usually he employs sodium sulfhydrate or arsenic trisulfide. In some cases, a mixture of arsenic trisulfide and sodium cyanide are used. For calf leather, the tanner usually makes use of paddles

and a short liming period. For making side leather, a "burning off" process may be used, *i.e.*, a very high sulfide-lime liquor, in which the hair is practically destroyed and more or less dissolved by the unhairing liquor. This type of liquor is the one generally used by the "Patent" leather tanner. However, for making certain types of side leather, conventional lime liquor, similar to that used for calf leather, may be utilized.

The origin of the use of alkalies, such as lime, and of sulfides, is unknown. It certainly dates back to very early times, however, since one of the stories in the "The Thousand Nights and a Night" describes the use of "a paste of yellow arsenic and quicklime" as a depilatory. The literature of the fifty years preceding 1925 contains many references to the various phases of the unhairing process. However, it remained for Stiasny[40] in 1906 to make the classical investigation of liming. In this study, he pointed out facts upon which most of present unhairing research rests. Stiasny was the first to suggest that unhairing was a function of the SH^- and OH^- ions. Wilson[51] and Merrill[31] in the early twenties started fundamental studies relative to this subject. Most of their work is adequately covered in Volume I of the second edition of this monograph, and will not further be elaborated upon here.

In 1925, McLaughlin and Theis[29] began a systematic investigation dealing with depilation. They studied in detail the effects of a straight lime upon such important factors as white weight gain, protein losses, effect of agitation and lime liquor changes and CaO absorption by the skin during the liming period. Their data indicated the following: (1) the liming requirements of Domestic and Frigorifico hides are different; (2) the greatest white weight comes in the early hours of liming; (3) this weight cannot be taken advantage of until the hair slips properly; (4) if liming is carried beyond the point of proper hair slippage, the white weight is reduced and the amount of dissolved hide substance increased; (5) maximum CaO absorption by the hide occurs in about 6 days; (6) temperature plays an important role, in that cold liquors delay unhairing, while warm liquors hasten this action; however, warm liquors reduce white weight gain and cause destruction of valuable hide substance; (7) a minimum amount of excess solid lime is necessary for proper conditioning of the hide; and (8) a ratio of one pound of hide to four pounds of lime liquor is preferable for proper unhairing.

In 1927, McLaughlin and Rockwell[26] studied the bacteriological phases of liming and came to the following conclusions: (1) fresh lime is able to unhair without aid from bacteria; (2) non-spore-forming bacteria are destroyed by fresh lime; (3) sporulating bacteria are not killed by a straight lime; (4) the disinfectant value of a lime liquor is governed by its alkalinity and by its ability to absorb the carbon dioxide necessary for bacterial growth; (5) bacteria may function in an old lime of reduced disinfectant power; and (6), the

liming process is essentially chemical and is governed to a high degree by the bacterial activity upon the hide or skin during curing and soaking.

Merrill[31] and Marriott[22] independently suggested a theory of depilation to the effect that unhairing is dependent on reducing agents active in alkaline solution. These reducing agents can arise from the breakdown of the keratinous substances of the skin or from the addition of other agents, *i.e.*, sulfides, cyanides, etc. The investigation made by Marriott in 1928 was of two-fold importance; first, he postulated many of the reactions taking place between the skin proteins and the specific unhairing liquor; secondly, his experimental findings were followed by a suggested theory applicable to the whole unhairing action. The work of Merrill and Marriott permits the following conclusions: (1) sodium sulfide reacts with the keratins of the hair through the medium of the disulfide linkage; (2) reducing agents active in alkaline solution actively increase the unhairing rate; (3) oxidizing agents retard depilation rate; (4) the power of the reducing substances to increase the unhairing rate is primarily dependent upon the OH^- ion concentration; (5) alkaline unhairing media containing no added reducing agent are dependent upon the OH^- ion concentration and the chemical ability of the media to produce it; (6) calcium hydroxide acts upon the hair in such a way as to produce $S^=$ or SH^- ions; (7) the hydrolysis of hair in the pH range 8 to 10.5 is probably not a simple function of the OH^- ion concentration; (8) calcium hydroxide suspensions act upon the cystine, breaking the —S—S— linkage, and produce metallic sulfides and ammonia; (9) the ammonia so formed is believed to increase with increasing sulfur content of the skin proteins; (10) reducing agents appear to delay breakdown of collagen; (11) the portion of the collagen acted upon by the alkaline unhairing liquor yields a large proportion of its total nitrogen as ammonia; and (12) the source of the ammonia appears to be the cystine linkages of the hair keratin and the acid-amide groups present both in the keratin and collagen.

McLaughlin and Theis in their early studies found that if no sulfide is present in the lime liquor only some 10 per cent of the total nitrogen is derived from the hair, and that this amount is not increased until the sulfide concentration becomes greater than 0.2 per cent solution. McLaughlin and Theis were able to demonstrate experimentally that about one-half the total nitrogen dissolved from the hide during the unhairing operation was derived from the flesh side of the hide.

Gustavson and Widen[11] state that the action of lime and alkalies in general upon protein may be: (1) formation of a chemical compound with the specific protein by means of primary valence, which, in turn, may lead to a splitting off of elementary groups, giving rise to an increase in the number of acidic and basic groups; (2) the salt formation may cause a redistribution and increase in the strength of the auxiliary forces; and (3) the aggre-

gates of elementary forces, which may possibly have formed through cohesion and other similar forces, are then broken up.

It has been stated previously that there are many modifications of the unhairing process and that various liquors are used in practice. Some of the more important ones will now be discussed in detail.

The Dimethylamine System

In 1927, McLaughlin, Highberger and Moore[25] published their studies of the use of methylamine in unhairing. This work was of considerable importance because for the first time one of the main constituents active in the so-called "mellow" limes was studied. Their data are shown in Tables 50-52 and Figures 37, 38, and 39. These data show that unhairing occurred at the

Table 50. Showing the Relation of Sulfur and Protein Nitrogen in the Lime Liquor to the Time of Unhairing of Fresh Steer Hide, when Limed in the Weight Proportion of 1 Part Hide to 4 Parts Solution, at 20° C (68° F).

Results expressed as gms/24 sq in of fresh hide (approx. 100 gms)

Period of liming in hours		Unhairing condition	Total sulfur	Sulfur by reduction evolution	Protein nitrogen
Lime only	24	+ to + +	0.0176	0.0018	0.0094
	36	+ to + +	0.0187	0.0019	0.0102
	48	+ + to + + +	0.0206	0.0021	0.0218
	60	+ + -	0.0196	0.0022	
	72	+ +	0.0243	0.0056	0.0189
	96	+ + +	0.0312	0.0080	0.0420
	120	+ + + to + + + +	0.0356	0.0086	0.0580
Lime + .32 *M* Methyl Amine	24	+ +	0.0166	0.0006	0.0173
	36	+ + to + + -	0.0177	0.0033	0.0229
	48	+ + + to + + + -	0.0280	0.0027	0.0339
	60	+ + +	0.0345	0.0045	0.0581
	72	+ + + +	0.0396	0.0066	0.0732
	96	+ + + + +	0.0313	0.0092	0.1107
	120		0.0515	0.0154	0.1362
Lime + .32 *M* Ethyl Amine	24	+ + -	0.0155	0.0007	0.0100
	36	+ +	0.0237	0.0015	0.0165
	48	+ + to + + -	0.0209	0.0010	0.0203
	60	+ + +	0.0268	0.0017	0.0378
	72	+ + + to + + + +	0.0325	0.0046	0.0479
	96	+ + + + +	0.0368	0.0080	0.0862
	120		0.0430	0.0121	0.1011
Lime + .32 *M* Trimethyl Amine	24	+ + -	0.0152	0.0000	0.0098
	36	+ + to + + +	0.0161	0.0006	0.0090
	48	+ + to + +	0.0192	0.0000	0.0124
	60	+ to + + -	0.0236	0.0000	0.0134
	72	+ +	0.0295	0.0004	0.0316
	96	+ + + -	0.0290	0.0032	0.0377
	120	+ + +	0.0298	0.0055	0.0520
Lime + .32 *M* Ammonia	24	+ + -	0.0130	0.0004	0.0120
	36	+ +	0.0172	0.0002	0.0138
	48	+ + -	0.0188	0.0000	0.0143
	60	+ + to + + -	0.0217	0.0003	0.0192
	72	+ +	0.0228	0.0004	0.0433
	96	+ + +	0.0240	0.0034	0.0491
	120	+ + + to + + + -	0.0271	0.0063	0.0736

Table 51. Showing the Relation of Sulfur and Protein Nitrogen in the Lime Liquor to the Time of Unhairing of Russian Dried (Unsalted) Goat Skin, when Limed in the Weight Proportion of 1 Part Dried Skin to 12 Parts Solution, at 20° C (68° F).

Results expressed as gms/35 sq in dried skin (27 to 37 gms)

Period of liming in hours		Unhairing condition	Total sulfur	Sulfur by reduction evolution	Protein nitrogen
	24	+ to −	.0768	.0480	.0302
Lime	48	+	.1024	.0475	.0585
only	72	+ +	.1402	.0530	.0873
	96	+ + + − to + +	.1694	.0895	.0983
	120	+ + +	.1755	.1020	.0951
	24	+ +	.1044	.0650	.0666
Lime	48	+ + +	.1766	.1090	.1358
+ .32 *M*	72	+ + + + −	.2141	.1315	.1061
Methyl	96	+ + + + +	.2302	.1390	.1302
Amine	120	very easy	.2920	.1695	.2002
	24	+ to + +	.1090	.0610	.0546
Lime	48	+ + + −	.1658	.0950	.0888
+ .32 *M*	60	+ + +	.1574	.0930	.1152
Ethyl	96	+ + + +	.2498	.1570	.1425
Amine	120	+ + + + +	.2471	.1510	.1429
	24	+ to −	.0874	.0320	.0435
Lime	48	+ to + +	.1284	.0615	.0652
+ .32 *M*	72	+ +	.1347	.0810	.0848
Trimethyl	96	+ + +	.1575	.0875	.1031
Amine	120	+ + + to + + + +	.1988	.1120	.1041
	24	+ to −	.0909	.0390	.0581
Lime	48	+ +	.1085	.0530	.0721
+ .32 *M*	72	+ +	.1481	.0815	.0990
Ammonia	96	+ + +	.1582	.0915	.0993
	120	+ + + to + + + +	.2304	.1270	.1270

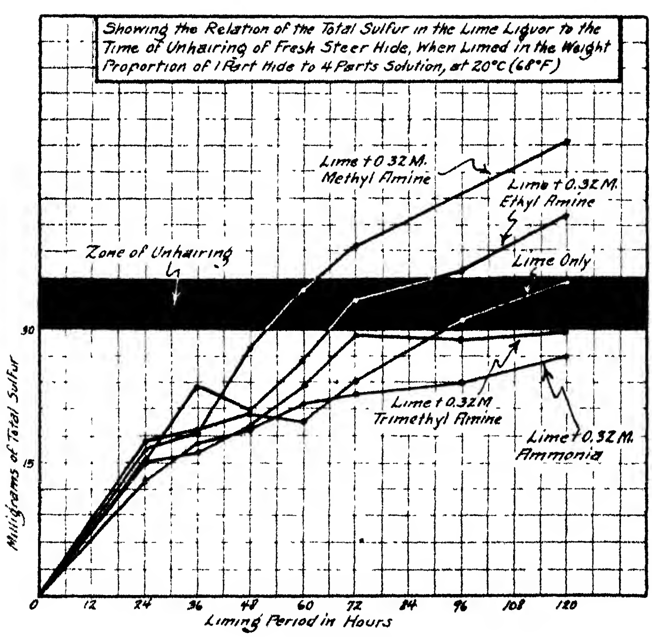


Figure 37

Table 52. Showing the Relation of Sulfur and of Protein Nitrogen in the Lime Liquor to the Time of Unhairing of Fresh Calf Skin, when Limed in the Weight Proportion of 1 Part Skin to 4 Parts Solution, at 20° C (68° F).

Results expressed as grams per 48 sq in of skin (approx. 90 gms)

Period of liming in hours		Unhairing condition	Total sulfur	Sulfur by reduction evolution	Protein nitrogen
Lime Only	24	+ −	0.0195	0.0038	0.0447
	48	+ to + +	0.0181	0.0092	0.0493
	72	+ +	0.0281	0.0126	0.0740
	96	+ + + −	0.0343	0.0174	0.0918
	108	+ + +	0.0426	0.0189	0.1232
Lime + 0.32 *M* Methyl Amine	24	+	0.0268	0.0087	0.0559
	48	+ + +	0.0434	0.0244	0.0717
	72	+ + + +	0.0588	0.0330	0.1208
	96	+ + + + +	0.0678	0.0344	0.1715
	108	very easy	0.0628	0.0377	0.1895
Lime + 0.32 *M* Ethyl Amine	24	+ to + −	0.0202	0.0114	0.0396
	48	+ + + −	0.0357	0.0187	0.0646
	72	+ + + to + + + + −	0.0468	0.0235	
	96	+ + + + +	0.0502	0.0283	0.1198
	108	very easy	0.0590	0.0299	0.1488
Lime + 0.32 *M* Trimethyl Amine	24	+ −	0.0076	0.0018	0.0284
	48	+ + −	0.0194	0.0073	0.0454
	72	+ +	0.0228	0.0111	0.0617
	96	+ + + −	0.0426	0.0230	0.0997
	108	+ + +	0.0441	0.0216	0.1090
Lime + 0.32 *M* Ammonia	24	+ −	0.0106	0.0025	0.0324
	48	+ to + + −	0.0180	0.0086	0.0651
	72	+ + to + + + −	0.0325	0.0152	0.1030
	96	+ + + −	0.0409	0.01[illegible]	0.1158
	108	+ + +	0.0481	0.0216	0.1482

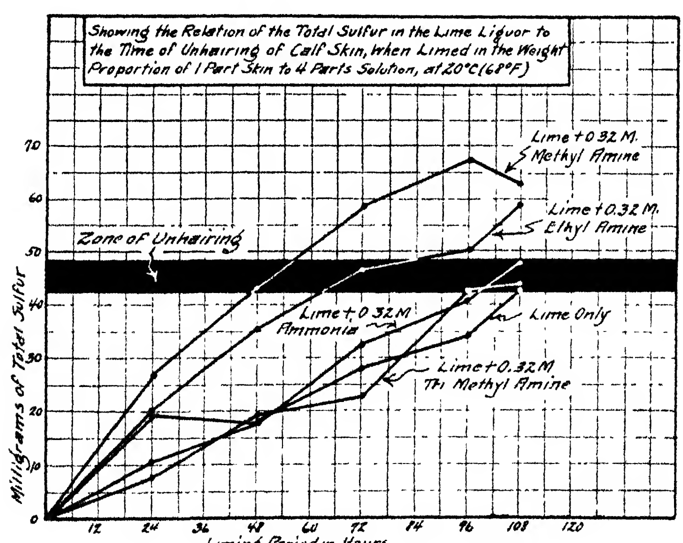
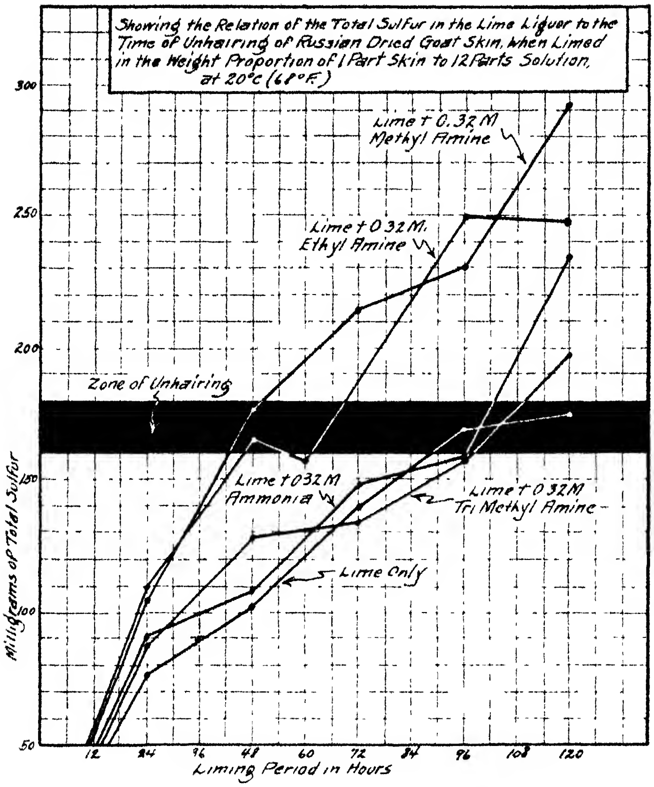


Figure 37—*Continued*


Showing the Relation of the Total Sulfur in the Lime Liquor to the Time of Unhairing of Russian Dried Goat Skin, when Limed in the Weight Proportion of 1 Part Skin to 12 Parts Solution, at 20°C (68°F.)
Lime + 0.32 M Methyl Amine
Lime + 0.32 M. Ethyl Amine
Zone of Unhairing
Lime + 0.32 M Ammonia
Lime + 0.32 M Tri Methyl Amine
Lime Only
Milligrams of Total Sulfur
Liming Period in Hours

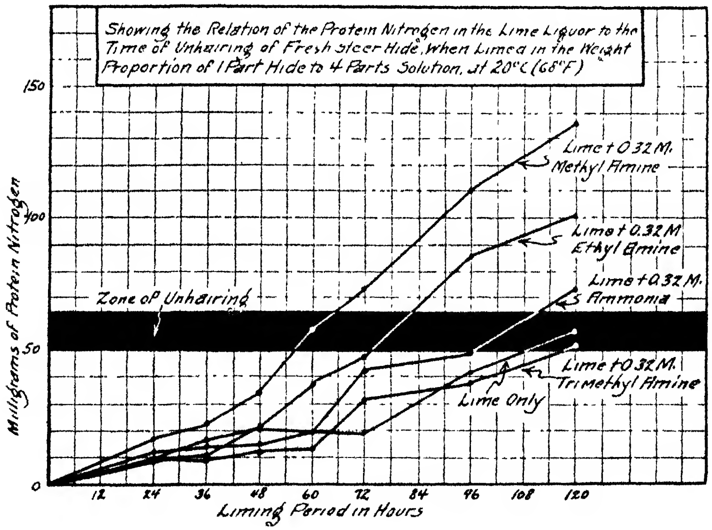
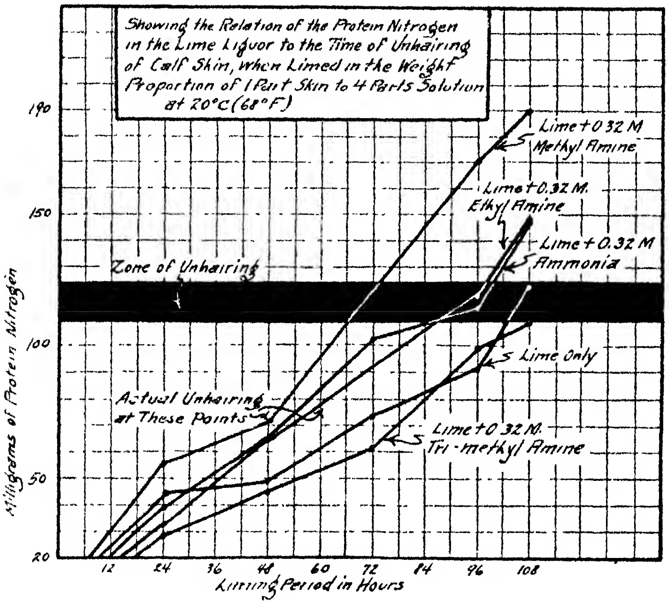

Showing the Relation of the Protein Nitrogen in the Lime Liquor to the Time of Unhairing of Fresh Steer Hide, When Limed in the Weight Proportion of 1 Part Hide to 4 Parts Solution, at 20°C (68°F)
Lime + 0.32 M. Methyl Amine
Lime + 0.32 M Ethyl Amine
Lime + 0.32 M. Ammonia
Zone of Unhairing
Lime + 0.32 M. Tri Methyl Amine
Lime Only
Milligrams of Protein Nitrogen
Liming Period in Hours

Figure 38


Showing the Relation of the Protein Nitrogen in the Lime Liquor to the Time of Unhairing of Calf Skin, when Limed in the Weight Proportion of 1 Part Skin to 4 Parts Solution at 20°C (68°F)
Lime + 0.32 M Methyl Amine
Lime + 0.32 M. Ethyl Amine
Lime + 0.32 M Ammonia
Zone of Unhairing
Lime Only
Actual Unhairing at These Points
Lime + 0.32 M. Tri-methyl Amine
Milligrams of Protein Nitrogen
Liming Period in Hours

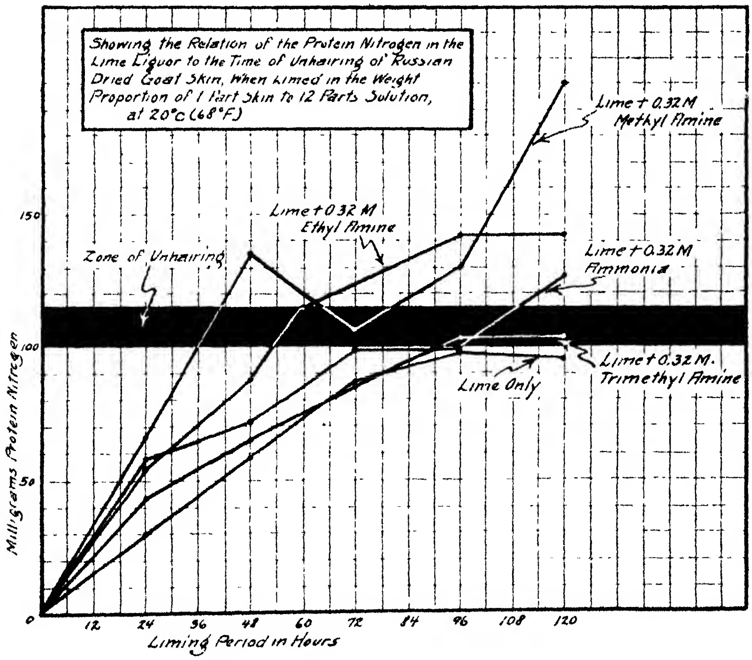

Showing the Relation of the Protein Nitrogen in the Lime Liquor to the Time of Unhairing of Russian Dried Goat Skin, When Limed in the Weight Proportion of 1 Part Skin to 12 Parts Solution, at 20°C (68°F)
Lime + 0.32 M Methyl Amine
Lime + 0.32 M Ethyl Amine
Zone of Unhairing
Lime + 0.32 M Ammonia
Lime + 0.32 M. Trimethyl Amine
Lime Only
Milligrams Protein Nitrogen
Liming Period in Hours

Figure 39

point + + + regardless of whether normal or accelerated unhairing was involved. These investigators pointed out that, regardless of the unhairing time, a certain minimal destruction of the epidermal keratin must take place and that this destruction can be measured by the sulfur content of the liming liquor. McLaughlin *et al.* in addition, pointed out that while primary alkyl amines greatly reduce the unhairing time, secondary and tertiary amines have little or no effect. It further was stated that the action of the amines was not due to increased solubility of the lime, nor to increased alkalinity, but was probably the result of direct chemical action.

Wilson, Merrill and Fleming investigated the unhairing action of methylamine and found it to be a much more powerful unhairing agent than sodium sulfide. In 1928, McLaughlin, Highberger and Moore studied the origin of the amines in lime liquor. These investigators developed a quantitative method for estimating amines in the skin itself and in the residual liquors after soaking and liming. That amines are present during the cure is shown in Table 53. McLaughlin *et al.* demonstrated the formation of amines during post mortem action, as shown in Table 54.

Table 53. The Volatile Amine Content of Brines from a Hide Cellar.

Source of brine	Grams nitrogen per 100 cc — Total volatile	Volatile amine
From heavy steer and cow. Longest cure 60 days.	0.0454	0.0043
From calf skins. Longest cure 3 months, shortest 5 hrs.	0.0457	0.0036
From sheep skins. Longest cure 2 days, shortest 1 day.	0.0347	0.0022
Pool on floor, drainings from heavy steer and cow hides. Pool about 18 hours old.	0.0247	0.0015

Table 54. The Post Mortem Production of Ammonia and Amine in Fresh Steer Hide at 20° C (68° F).

Hours elapsing from death of animal	Grams nitrogen per 100 grams hide on flayed weight — Ammonia	Volatile amine
1	0.1962	0.0062
4	0.2045	0.0051
9	0.2113	0.0065
22	0.2084	0.0052
30	0.2064	0.0076
46	0.2178	0.0086
59	0.2436	0.0116
72	0.2767	0.0133

Since post mortem changes taking place within the skin reflect themselves in subsequent processes, the formation of amines during the period before curing and in curing itself is an important factor. Table 55 shows that the formation of ammonia and amines parallel bacterial activity; hence the type and period of soaking become increasingly important.

McLaughlin *et al.* state that since amines are formed predominantly as a

result of bacterial action, their formation during the liming process will be less, since bacterial activity is almost nil in this process. Table 56 shows this statement to be true.

Table 55. The Production of Ammonia and Amines in Domestic Steer Hide and in the Soak Water, After Soaking 72 Hours at Various Temperatures, in the Proportion of 1 Part Hide to 4 Parts Water.

Temp. of soak (° C)	(° F)	Expt. No.	Grams nitrogen per 100 grams cured hide — In hide — Ammonia	Volatile amine	In soak water — Ammonia	Volatile amine
		1	0.2496	0.0029	0.0760	0.0048
		2	0.2567	0.0036	0.0612	0.0028
15	59	3	0.2415	0.0028	0.0732	0.0024
		4	0.2544	0.0036	0.0828	0.0040
		5	0.2450	0.0025	0.0804	0.0036
		1	0.2349	0.0026	0.0856	0.0056
		2	0.2758	0.0047	.....	.....
20	68	3	0.2966	0.0049	0.0792	0.0032
		4	0.2496	0.0032	. .	. . . .
		5	0.2472	0.0027	0.0860	0.0032
		6	0.2623	0.0029	0.0836	0.0036
		1	0.3104	0.0037	0.2876	0.0084
		2	0.3049	0.0036	0.1560	0.0060
30	86	3	0.2095	0.0025	0.3004	0.0064
		4	0.3132	0.0048	0.1856	0.0048
		5	0.3318	0.0077	0.3208	0.0056

Table 56. The Production of Ammonia and Amines in the Lime Liquor When Frigorifico Steer Hides are Limes in the Proportion of 1 Part Hide to 4 Parts Solution at 20° C (68° F), After Soaking 18 Hours at the Same Temperature and Proportion.

Hours in lime	Grams nitrogen of the lime liquor per 100 grams of hide — Ammonia	Volatile amine
12	0.0130	0.0006
24	0.0162	0.0006
36	0.0242	0.0006
48	0.0278	0.0014
72	0.0374	0.0018

In 1932, Moore, Highberger and O'Flaherty[35] studied the effects of mono-, di-, and trimethylamines and mixtures of these upon unhairing. Their data are shown in Tables 57 to 61. These data indicate that dimethylamine accelerates unhairing as well as, if not better than the mono- derivative; that mixtures of the mono- and dialkylamines possess additive acceleration powers; that trimethylamine has but little unhairing effect; that dimethylamine in high concentration markedly affects the hair; and that the amine concentration used in practise is without harmful effect on the hide or hair.

In 1930, Bergmann[4] patented a process for unhairing hides and skins. In this patent, he pointed out the possible effect of such substances as the organic nitrogen bases such as ammonia, ethylenediamine, piperidine, pyri-

(Text continued on page 183)

Table 57. Action of Lime Suspensions Containing Methylamine, Dimethylamine or Trimethylamine on Frigorifico Cured Steer Hide.

Kind of amine	Concentration Molar	Concentration G per 100 cc	Hours in limes	Mg non-volatile nitrogen in limes from 100 g soaked, fleshed hide	Unhairing condition	G limed, unhaired fleshed hide from 100 g soaked, fleshed hide	Apparent action on hair
None	0.00	0.00	96	90	+ + +	96	none
None	0.00	0.00	96	92	+ + +	86	none
None	0.00	0.00	96	144	+ + +	86	none
None	0.00	0.00	96	88	+ + +	84	none
None	0.00	0.00	96	124	+ + +	88	none
None	0.00	0.00	120	126	+ + +	97	none
				Ave. 110		Ave. 89	
Methylamine	0.32	1.00	41	76(?)	+ − +	92	none
Methylamine	0.16	0.50	41	108	+ + +	86	none
Methylamine	0.08	0.25	66	104	+ + +	97	none
Dimethylamine	0.32	1.44	41	130	+ + +	92	marked
Dimethylamine	0.16	0.72	41	114	+ + +	92	moderate
Dimethylamine	0.08	0.36	48	100	+ + +	92	none
Trimethylamine	0.32	1.89	96	116	+ + +	87	none
Trimethylamine	0.16	0.94	120	120	+ + +	97	none
Trimethylamine	0.08	0.47	96	110	+ + +	91	none

Apparent condition of limed hide good in all cases.

Note: + + + indicates satisfactory unhairing.

Table 58. Action of Lime Suspensions Containing Both Methylamine and Dimethylamine on Frigorifico Cured Steer Hide.

Methylamine Molar conc	Methylamine G per 100 cc	Dimethylamine Molar conc	Dimethylamine G per 100 cc	Hours in limes	Mg non-volatile nitrogen in limes from 100 g soaked, fleshed hide	Unhairing condition	G limed, unhaired, fleshed hide from 100 g soaked, fleshed hide	Apparent action on hair
0.32	1.00	0.32	1.44	41	180	+ + + +	86	marked
0.32	1.00	0.16	0.72	41	134	+ + + to + + + +	92	moderate
0.32	1.00	0.08	0.36	41	128	+ + + +	92	slight
0.32	1.00	0.00	0.00	41	76(?)	+ + +	92	none
0.16	0.50	0.32	1.44	41	146	+ + + to + + + +	88	very marked
0.16	0.50	0.16	0.72	48	150	+ + + +	96	marked
0.16	0.50	0.08	0.36	41	134	+ + +	86	slight
0.16	0.50	0.00	0.00	41	108	+ + +	86	none
0.08	0.25	0.32	1.44	41	140	+ + + +	91	moderate
0.08	0.25	0.16	0.72	41	140	+ + + +	92	none
0.08	0.25	0.08	0.36	41	134	+ + +	102(?)	none
0.08	0.25	0.00	0.00	66	104	+ + +	97	none
0.00	0.00	0.32	1.44	41	130	+ + +	92	marked
0.00	0.00	0.16	0.72	41	114	+ + +	92	moderate
0.00	0.00	0.08	0.36	48	100	+ + +	92	none
0.00	0.00	0.00	0.00	96 to 120	88 to 144, (average 110)	+ + +	84 to 96, (average 89)	none

Apparent condition of limed hide good in all cases.

Note: + + + indicates satisfactory unhairing.
+ + + + indicates easier unhairing than + + +.

Table 59. Action of Lime Suspensions Containing Both Methylamine and Trimethylamine on Frigorifico Cured Steer Hide.

Methylamine		Trimethylamine						
Molar conc	G per 100 cc	Molar conc	G per 100 cc	Hours in limes	Mg non-volatile nitrogen in limes from 100 g soaked, fleshed hide	Unhairing condition	G limed, unhaired, fleshed hide from 100 g soaked, fleshed hide	Apparent action on hair
0.32	1.00	0.32	1.89	41	116	+ + +	92	none
0.32	1.00	0.16	0.94	41	130	+ + +	88	none
0.32	1.00	0.08	0.47	48	126	+ + + +	102	none
0.32	1.00	0.00	0.00	41	76(?)	+ + +	92	none
0.16	0.50	0.32	1.89	66	86	+ + +	92	none
0.16	0.50	0.16	0.94	66	126	+ + +	88	none
0.16	0.50	0.08	0.47	48	100	+ + +	87	none
0.16	0.50	0.00	0.00	41	108	+ + +	86	none
0.08	0.25	0.32	1.89	72	108	+ + +	92	none
0.08	0.25	0.16	0.94	72	88	+ + +	92	none
0.08	0.25	0.08	0.47	66	82	+ + +	92	none
0.08	0.25	0.00	0.00	66	104	+ + +	97	none
0.00	0.00	0.32	1.89	96	116	+ + +	87	none
0.00	0.00	0.16	0.94	120	120	+ + +	97	none
0.00	0.00	0.08	0.47	96	110	+ + +	91	none
0.00	0.00	0.00	0.00	96 to 120	88 to 144 (average 110)	+ + +	84 to 96 (average 89)	

Apparent condition of limed hide good in all cases.

Note: + + + indicates satisfactory unhairing.
+ + + + indicates easier unhairing than + + +.

Table 60. Action of Lime Suspensions Containing Both Dimethylamine and Trimethylamine on Frigorifico Cured Steer Hide.

Dimethylamine Molar conc.	Dimethylamine G per 100 cc	Trimethylamine Molar conc.	Trimethylamine G per 100 cc	Hours in limes	Mg non-volatile nitrogen in limes from 100 g soaked, fleshed hide	Unhairing condition	G limed, unhaired, fleshed hide from 100 g soaked, fleshed hide	Apparent action on hair
0.32	1.44	0.32	1.89	48	108	+ + + to + + + −	93	marked
0.32	1.44	0.16	0.94	41	142	+ + +	87	marked
0.32	1.44	0.08	0.47	41	152	+ + +	92	marked
0.32	1.44	0.00	0.00	41	130	+ + +	92	marked
0.16	0.72	0.32	1.89	48	122	+ + + −	86	none
0.16	0.72	0.16	0.94	41	152	+ + +	86	none
0.16	0.72	0.08	0.47	41	112	+ + +	87	none
0.16	0.72	0.00	0.00	41	114	+ + +	92	none
0.08	0.36	0.32	1.89	66	96	+ + + +	92	none
0.08	0.36	0.16	0.94	66	126	+ + + +	89	none
0.08	0.36	0.08	0.47	48	98	+ + +	95	none
0.08	0.36	0.00	0.00	48	100	+ + +	92	none
0.00	0.00	0.32	1.89	96	116	+ + +	87	none
0.00	0.00	0.16	0.94	120	120	+ + +	97	none
0.00	0.00	0.08	0.47	96	110	+ + +	91	none
0.00	0.00	0.00	0.00	96 to 120	88 to 144 (average 110)	+ + +	84 to 96 (average 89)	

Apparent condition of limed hide good in all cases.

Note: + + + − indicates not quite satisfactory unhairing.
+ + + indicates satisfactory unhairing.
+ + + + indicates easier unhairing than + + +.

Table 61. Action of Lime Suspensions Containing Methylamine, Dimethylamine and Trimethylamine on Frigorifico Cured Steer Hide.

Methylamine Molar conc	Methylamine G per 100 cc	Dimethylamine Molar conc.	Dimethylamine G per 100 cc	Trimethylamine Molar conc	Trimethylamine G per 100 cc	Hours in limes	Mg non-volatile nitrogen in limes per 100 g soaked, fleshed hide	Unhairing condition	G limed, unhaired, fleshed hide from 100 g soaked, fleshed hide	Apparent action on hair
0.32	1.00	0.32	1.44	0.32	1.89	48	122	+ + + −	98	marked
0.32	1.00	0.32	1.44	0.08	0.47	41	140	+ + +	91	marked
0.32	1.00	0.16	0.72	0.16	0.94	41	142	+ + + +	93	marked
0.32	1.00	0.08	0.36	0.32	1.89	48	96	+ + +	100	none
0.32	1.00	0.08	0.36	0.08	0.47	48	112	+ + + +	95	none
0.32	1.00	0.00	0.00	0.00	0.00	41	76(?)	+ + +	92	none
0.16	0.50	0.32	1.44	0.32	1.89	41	138	+ + +	91	marked
0.16	0.50	0.32	1.44	0.08	0.47	41	148	+ + +	93	slight
0.16	0.50	0.16	0.72	0.16	0.94	41	100	+ + +	86	marked
0.16	0.50	0.08	0.36	0.32	1.89	48	98	+ + +	90	none
0.16	0.50	0.08	0.36	0.08	0.47	48	110	+ + +	92	none
0.16	0.50	0.00	0.00	0.00	0.00	41	108	+ + +	86	none
0.08	0.25	0.32	1.44	0.32	1.89	48	110	+ + + −	98	none
0.08	0.25	0.32	1.44	0.08	0.47	41	148	+ + + +	93	moderate
0.08	0.25	0.16	0.72	0.16	0.94	48	88	+ + +	92	none
0.08	0.25	0.08	0.36	0.32	1.89	66	120	+ + + +	101	none
0.08	0.25	0.08	0.36	0.08	0.47	41	102	+ + + to + + + +	97	none
0.08	0.25	0.00	0.00	0.00	0.00	66	104	+ + +	97	none
0.00	0.00	0.00	0.00	0.00	0.00	96 to 120	88 to 144 (average 110)	+ + +	84 to 96 (average 89)	none

Apparent condition of limed hide good in all cases.

Note: + + + − indicates not quite satisfactory unhairing.
+ + + indicates satisfactory unhairing
+ + + + indicates easier unhairing than + + +.

dine, and ureas, and especially the sulfides of such bases. Also, in 1930, McLaughlin, Rockwell, O'Flaherty and Highberger[27] patented the use of amines specifically as accelerating unhairing agents.

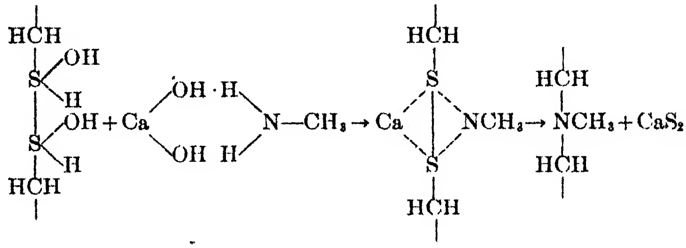
The general effect of the mono- and dimethylamines in accelerating unhairing is not shared by ammonia and appears to be at variance with the theory of depilation advanced by Merrill and Marriott, namely, that depilation in alkaline solution depends upon reducing agents, since amines do not possess reducing powers. Phillips attempted to link up the amine effect with the reduction theory by postulating that, after the sulfur groupings had been hydrated in the presence of alkali, autooxidation-reduction of the sulfenic acid side chain causes the formation of hydrogen sulfide and of an aldehyde group. Marriott maintains that this theory cannot explain the vigorous unhairing effect of dimethylamine. In 1937, Marriott[23] investigated the effect of alkyl amines upon cystine and goat hair. From this study, Marriott suggests that while the exact mechanism of the amine reaction may be obscure, it depends fundamentally upon the way in which the specific amine affects the formation of sulfur-containing reducing agents from the keratin. He maintains that the sulfur atoms are not present in keratin in the form of a simple cystine grouping. Marriott studied the reaction of the amines upon cystine and keratin in an environment of calcium hydroxide and sodium hydroxide. His results are shown in Table 62

Table 62. Action of Alkalies on Cystine and Hair and the Effect of Additions of Amines.

	"SH" present after					
Solution	1 day	2 days	3 days	4 days	5 days	8 days
Suspension of Lime						
+ cystine + $H_2N\ CH_3$	+ +	+ + + +	+ + + +	+ + + + +	+ + + + +	+ + + + +
+ cystine + $H\ N(CH_3)_2$		−		−	+	−
+ cystine + $N\ (CH_3)_3$		− +	+ +	+ +	+ + +	+ +
+ cystine	−	− +	+ +	+ +	+ + +	+ +
+ hair + $H_2N\ CH_3$	+	+ +	+ +	+ + +	+ +	+ + +
+ hair + $H\ N\ (CH_3)_2$	+ +	+ + + +	+ + + + +	+ + + + +	+ + + + +	+ + + + +
+ hair + $N\ (CH_3)_3$	−	+	+	−	+ +	+
+ hair	−	+	+	+	+ +	+
N/10 Sodium hydroxide						
+ cystine + $H_2N\ CH_3$	−			−	−	−
+ cystine + $H\ N\ (CH_3)_2$	−	−	−		−	−
+ cystine + $N\ (CH_3)_3$	−	−	−	−	−	−
+ cystine	−	−	−	−	−	−
+ hair + $H_2N\ CH_3$	+	+ +	+ +	+ +	+ +	+ +
+ hair + $H\ N\ (CH_3)_2$	+ + +	+ + + +	+ + + + +	+ + + + +	+ + + + +	+ + + + +
+ hair + $N\ (CH_3)_3$	− +	+	+	+	+	+
+ hair	− +	+	+	+	+	+

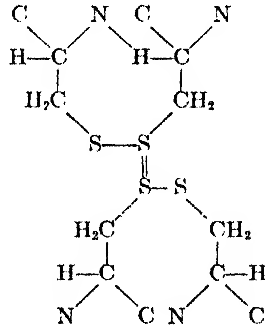
Marriott found in the lime-plus-cystine system that the addition of dimethylamine retards the production of sulfhydrate ion, that the addition of trimethylamine is without effect, and that the primary amine strongly affects the system. When hair is used in place of cystine, the secondary

amine far surpasses the primary amine in its action upon the keratin. Sodium hydroxide was found to act differently from calcium hydroxide, since in no case did any of the amines react with the cystine. However, when hair was used, the reaction was similar to that when lime was employed. From these observations, Marriott concluded that the sulfur in hair is not in the same form as the sulfur in the amino acid cystine. Marriott pictured the reaction of methylamine upon cystine somewhat as follows:



For dimethylamine, Marriott maintained that the presence of only one replaceable hydrogen prevents any combination with cystine and thus any breakdown of the S—S linkage to form polysulfide. As it has no replaceable hydrogen atoms, trimethylamine should be inert.

Many investigators have found that the keratin molecule is broken down by even mild alkali. Therefore, Marriott concludes that the sulfur in this particular molecule cannot be bound in the form of simple cystine groups. He pictures the cross linkages between and along the polypeptide chains of the keratin molecule somewhat after the pattern for polysulfides.



Marriott postulates that this particular configuration allows one of the tetravalent sulfur atoms to behave differently from the other three, thus enabling one out of every four to be removed by alkali. In strong alkali a scission of the two tetravalent sulfur atoms may occur, leaving a C—S—C linkage. Marriott thus concludes that the unhairing action of mono- and

dimethylamine is explained as being due indirectly to the formation of certain reducing groups.

In practice, methylamine was first employed, but was later replaced by the dimethylamine. The use of this substance yields a leather with a very fine grain; but generally, unless special precautions are taken, the leather may be flat and not have the desired fullness. For this reason, American practice in general is to use dimethylamine in conjunction with other unhairing aids, such as sodium sulfide, sodium sulfhydrate or sodium cyanide. The use of these unhairing adjuncts aids plumping of the hides or skins during the depilation period.

The Sulfide System

Some form or other of the lime-sulfide system is the one most generally used for the depilation of hides and skins in American practice. It may be that the tanner chooses sodium sulfide, sodium sulfhydrate, calcium sulfhydrate or arsenic sulfide for his purpose. Regardless of choice, the same general mechanism of reaction is involved.

The chemist's interest in the keratins is rather a negative one, since he always attempts to remove keratin material previous to tannage. Yet, in the main, the chemical reactions taking place during the unhairing operation are with the keratins of the skin.

The usual characterization of a keratin is that it is a protein making up the great bulk of such substances as epidermis, hair, wool, horn, nail, and feathers, and is outstanding in its content of sulfur. Block and Vickery[5] state: "A keratin is a protein which is resistant to digestion by pepsin and trypsin, which is insoluble in dilute acids and alkalies, in water and in organic solvents and which on acid hydrolysis yields such quantities of histidine, lysine and arginine that the molecular ratios of these amino acids are, respectively, approximately 1:4:12." They state further that a high proportion of cystine is not necessarily characteristic of the keratin protein. Table 63, taken from the work of Block and Vickery, gives the basic amino-acid content of various keratins.

Table 63. Basic Amino Acids of Various Keratins.

Protein	Arginine %	Histidine %	Lysine %	Cystine %
Human hair	8.0	0.5	2.5	16.5
Sheep wool	7.8	0.66	2.3	10.0
Snake epidermis	5.4	0.48	1.4	5.3
Goose feathers	4.8	0.35	1.04	6.4
Gorgonia flabellum	6.4	0.48	2.75	5.5
Plexaurella dichotoma	5.4	0.43	3.00	3.2
Silk fibroin	0.74	0.077	0.25	0.0

Michaelis[32] compares the keratin proteins with silk and cellulose with special relation to their colloidal properties. Silk thread is a chain of amino acid molecules. These threads of molecules are oriented parallel to one another and are linked together by some such agency as Van der Waals' forces or secondary valence. Keratin has a fibrous pattern, but it seems that the elementary fibers of keratin are held together not only by secondary valency forces but also by true chemical bonds. In contradistinction to silk and collagen fibers, the keratin fiber is said not to have a "shrinkage temperature." Keratins can be stretched, and this property may vary with the various forms of keratin. Astbury[1] distinguishes two forms of keratin, α and β. The former can be stretched only slightly, thus suggesting the idea that this particular keratin fiber has been stretched almost to a maximum. The β-keratins, found in wool and hair, can be stretched. Astbury maintains that the elementary fiber in its original pattern is not fully extended, but forms a zig-zag structure.

The bridging or linkage between elementary keratin fibers may be of several kinds: sulfur linkage due to the amino acid cystine; salt linkage due to the dicarboxylic and dibasic amino acids; and finally the short link (N—C), such as would be formed in practically any protein.

Keratin is very resistant to chemical and to enzymatic action. Acid decomposes it, reducing the protein to its constituent amino acids. Strong alkalies accomplish the same purpose, but alter the amino acid cystine. However, certain reducing agents react with the sulfur linkage, cleaving the elementary fibers from one another without destruction of the protein. The type reaction is:

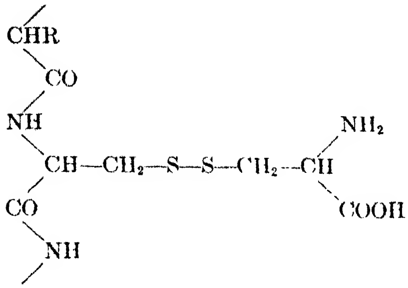
$$R—S—S—R + H_2 \rightarrow 2RSH$$

Michaelis indicated six different reductions of this sulfur linkage:

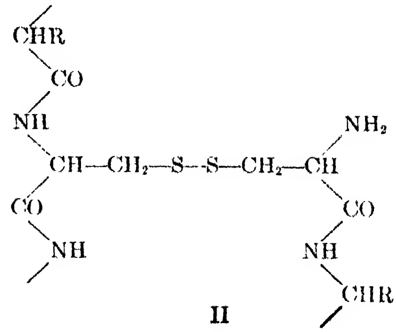
(1) $R—S—S—R + \text{(nascent) } H_2 \rightarrow 2RSH$
(2) $R—S—S—R + 2HS\ CH_2COOH \rightarrow 2RSH + (S\ CH_2\ COOH)_2$
(3) $R—S—S—R + HCN \rightarrow RSH + RSCN$
(4) $R—S—S—R + 2H_2S \rightarrow 2RSH + H_2S_2$
(5) $R—S—S—R + H_2S \rightarrow RSH + HS\ SR$
(6) $R—S—S—R + H_2SO_3 \rightarrow RSH + RS\ SO_3H$

Reaction (2) is the interesting one, since keratin is dissolved by thioglycollic acid at pH 12.0. It further appears from the analysis of the dissolved protein that only the —S—S— linkage of the protein has been disturbed; these change to —SH groups. Goddard and Michaelis[10] showed the changes taking place by use of thioglycollic acid, cyanide and sulfide.

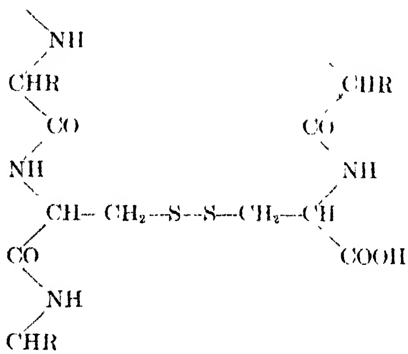
Harris and his co-workers[7, 12] in a recent series of papers have investigated the role played by cystine in the structure of wool. They indicate four possible structures for wool cystine:



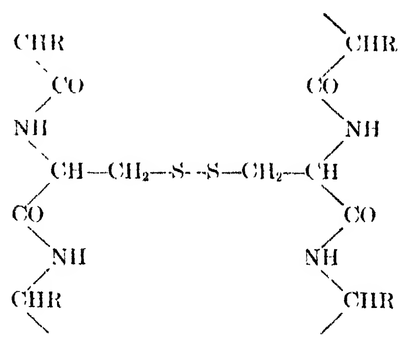
I



II



III



IV

It is pointed out that if the cystine of wool were combined in the peptide chain only through amino and carboxyl groups attached to the same carbon atom (formula I), any process breaking the disulfide linkage would eliminate one-half the sulfur. If formula II were correct, treatment with nitrous acid would destroy at least half the cystine. Titration curves of wool proteins indicate that all the carboxyl groups are accounted for by the content of glutamic and aspartic acids, more or less ruling out formula III. Thus the major part of the cystine must form parts either of two separate elementary fibers or of a single folded chain, as shown in formula IV.

Patterson, Geiger, Mizell and Harris[37] studied the effect of thioglycollic acid over a wide pH range and postulate:

$$\mathrm{W{-}S{-}S{-}W + 2HS{-}CH_2{-}COOH \rightarrow 2W{-}SH + (S{-}CH_2{-}COOH)_2} \qquad (1)$$

$$\mathrm{W{-}SH + RX \rightarrow W{-}SR + HX} \qquad (2)$$

where W represents wool connected by the disulfide grouping, R an alkyl group and X a halogen atom. The use of a dihalide gives

$$\mathrm{2W{-}SH + (CH_2)_nH_2 \rightarrow W{-}S{-}(CH_2)_2{-}S{-}W + 2HS} \qquad (3)$$

Reactions (2) and (3) offer a means of preparing a large number of wool derivatives.

Such reactions as occur in equations (1), (2) and (3) in the case of wool can well be applied to hair, and to studying the action of unhairing solutions upon the keratins of animal skin. Patterson *et al.* found that while the alkali-solubility of the reduced and methylated wool increased as its content of unchanged cystine decreased, that of wool reduced and treated with an alkyl dibromide decreased. It is further pointed out that such evidence indicates a rebuilding of cross-links of a type that are stable to alkali.

Schöberl and Eck,[39] in their study of the alkaline cleavage of disulfides, believe the following reaction occurs:

$$R—CH_2—S—S—CH_2—R \rightarrow R—CH_2—SH + HOSCH_2R$$
$$R—CH_2 \quad SOH \rightarrow RCHO + H_2S$$

Crowder and Harris[7] point out that their investigation indicates that the alkali degradation of wool proceeds according to the above equations, in that the alkali-treated samples showed "aldehyde" by Schiff's reagent. These workers also demonstrated that the sulfur split off is inorganic in character. Fig. 40, plotted from their data, shows the cystine loss and weight loss during treatment with 0.05*N* sodium hydroxide solution at 65° C. These data indicate a rapid splitting off of a portion of the sulfur during the early period of treatment. After 4 hours' treatment, the change is insignificant. These workers point out that the extreme case, in which approximately one-half the sulfur is removed, suggests the cleavage of the disulfide linkage into one labile and one comparatively stable sulfur atom. They further point out that under the conditions of the experiment cysteine and its derivatives would be stable, since the sulfur of the cysteine in wool (obtained by reduction of wool in alkaline solution) is stable under these conditions. They also suggest that since wool is not in solution, not all the —S—S— groups are reacted upon by the alkali, and that therefore it is probable that the sodium sulfide formed by the action of alkali upon some of the —S—S— groups tends to reduce the unreacted —S—S— grouping to sulfhydryl compounds, and thus increase the stability toward alkali.

Harris and Smith,[12] discussing the state of the sulfur in oxidized wool, showed the formation of the inorganic sulfur

$$\begin{tikzcd} R—CH_2—S—S—CH_2—R \arrow[r, shift left, "\text{Increasing pH}"] & R—CH_2—SH \arrow[l, shift left, "\text{Decreasing pH}"] \arrow[d, "\text{Decreasing pH}"'] & + & R—CH_2—SOH \\ & PbS & H_2S \arrow[l] & + \quad R—CHO \arrow[u, "\text{Increasing pH}"'] \end{tikzcd}$$

Since wool upon treatment with alkali tends to lose half of its sulfur quickly and then becomes very resistant to alkali, and since treatment with sodium sulfide increases the activity of the alkali on the keratin, it would

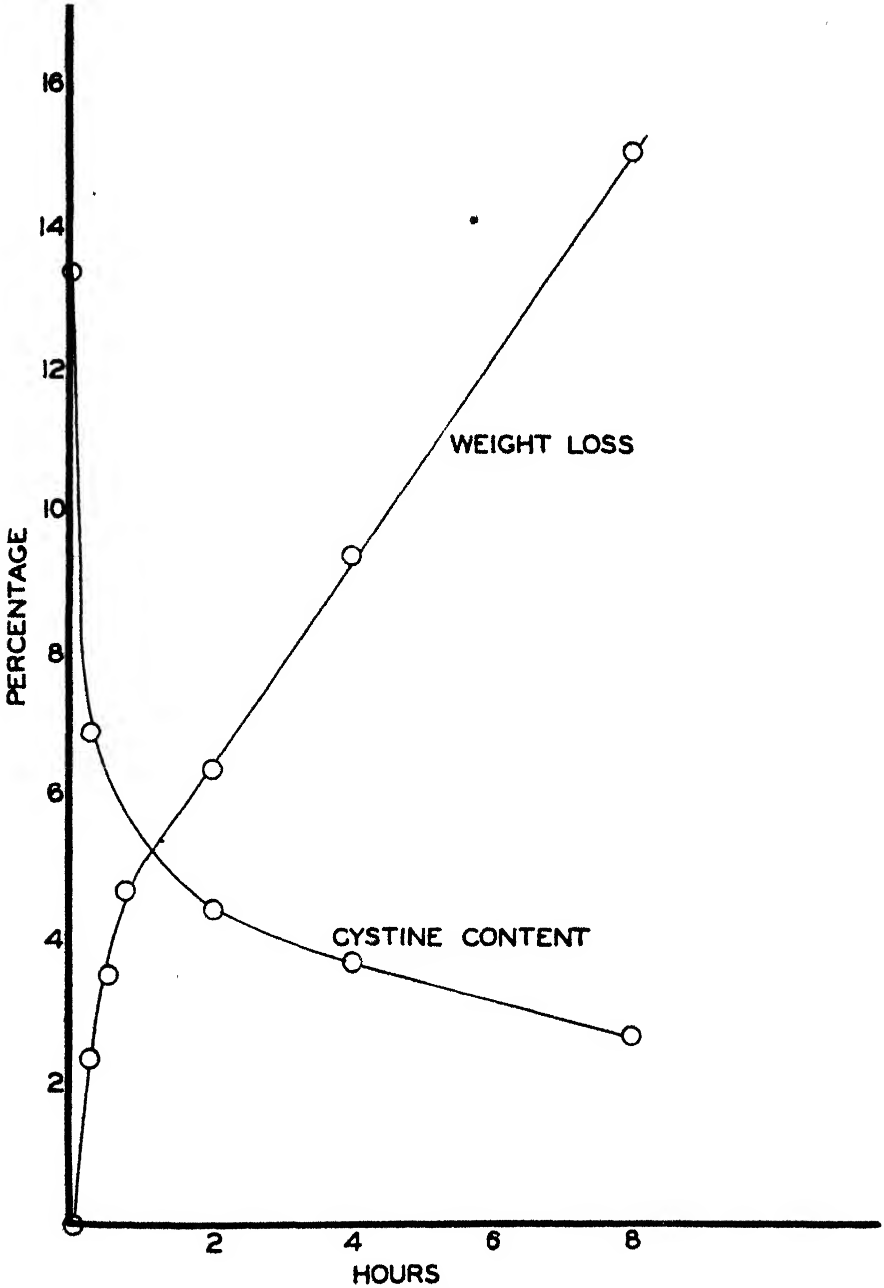


Figure 40. Cystine loss during treatment with sodium hydroxide (Crowder and Harris).

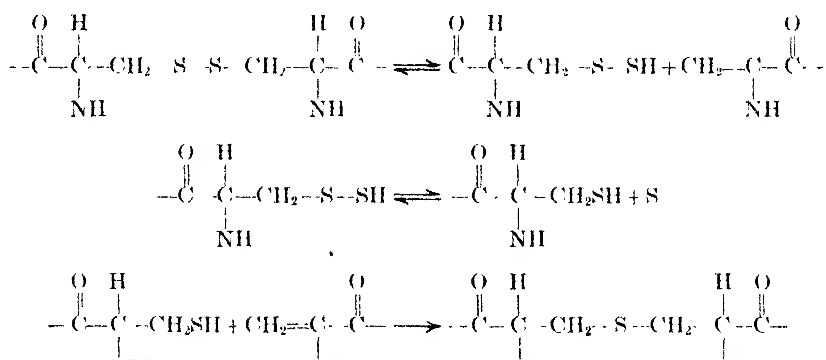
seem that the alkali really causes a change in the disulfide linkage such, perhaps, as the following:

$$R-S-S-R \rightarrow R-S-R + Na_2S$$

If such is the case, the R—S—R structure is much more resistant to alkali action. Hirsch,[15] in discussing the action of alkali on keratin, maintains that the alkali reacts on the disulfide linkage, changing it as postulated above. He further claims the R—S—R type of linkage is extremely resistant to strong sulfide solutions. This contention is borne out in practice, as will be discussed later. Hirsch calls this action "immunization" and claims that it begins at pH 12.0.

In 1943, Mizell and Harris[33] made a further study of the nature of the reaction of wool keratin with alkali. They point out that their results lead to the conclusion that the alkali cleavage of the disulfide group does not consist of a simple hydrolytic rupture of the —S—S— linkage, with subsequent formation of a sulfhydryl compound and a sulfenic acid, but involves rather a rupture between sulfur and carbon, yielding dehydroalanine and a $-CH_2-S-SH$ residue. This same mechanism was recently suggested by Nicolet and Shinn.[36]

Mizell and Harris point out that there is considerable support for the view that new linkages can be formed from ruptured ones, as has been postulated by Horn, Jones and Ringel.[16] Nicolet and Shinn[36] have postulated the following reaction, showing a split between carbon and sulfur:



cysteine *dehydroalanine* *lanthionine*

The above concept has many advantages over those of other investigators, since it not only explains the loss of half the sulfur but it meets the requirements for linkage reforming, and thus accounts for the relatively high tensile strength and low alkali-solubility of alkali-treated wool and hair. This view also substantiates the claim of Hirsch that a —C—S—C linkage is formed, bringing about immunization of the hair.

Theis and Ricker[47] made a study of the reactions taking place in the unhairing process. Their experimental procedure was the following. Thoroughly dry, green-salted, well fleshed cow hide was ground to a powder in a Wiley mill. The ground hair, hide and salt (contained in the cured hide) was allowed to air-condition for several days and was then thoroughly mixed. The mixed material was then analyzed for moisture, with the finding that 30 grams of this mixed material represented 50 grams of the original cured hide (containing 40 per cent moisture).

Six 30-gram samples of this special powder were soaked in water for 24 hours, filtered and thoroughly washed. Each of the six samples was then placed in 300 ml of 0.2*N* NaOH containing 0.5 gram of 70 per cent sodium sulfhydrate. The hide powders were subjected to this treatment for time intervals varying between zero and 170 hours. After definite intervals, the alkali-treated hide powder was filtered off and well washed, the solution and washings being carefully saved for analysis These solutions were analyzed for sulfite, sulfide, thiosulfate, polysulfide, and sulfate. The efficiency of recovery in terms of sulfur was about 95 per cent. The analytical methods used are given below.

The solution to be analyzed is filtered through a plug of absorbent cotton and washed with water until one liter of filtrate and washings have been collected in a volumetric flask. Aliquot portions of this solution are immediately treated as follows:

Total Sulfur: Dilute the sample with an equal volume of water, and oxidize with Br_2 by heating on a steam bath. Complete oxidization is assured and organic matter destroyed by fuming with a mixture of nitric and perchloric acids The resulting solution is neutralized with ammonia and rendered faintly acid with HCl, after which the sulfate is precipitated as $BaSO_4$, filtered, dried, and weighed.

Sulfide: The sulfide is removed from the solution by treatment with a $ZnCO_3$ suspension. The $ZnCO_3$—ZnS is filtered, washed, and dissolved in NaOH. Br_2 is used to oxidize the sulfide, the solution being heated on the steam bath for one hour. After neutralizing with HCl and adding a slight excess, the residual Br_2 is boiled off and the sulfate precipitated with $BaCl_2$. A blank must be deducted from the weight of $BaSO_4$ so obtained, since most $ZnCO_3$ contains some sulfur

Polysulfide: The sample is pipetted into a solution of KCN and boric acid, to form KCNS. Excess HCN is removed by vigorous boiling for 15-20 minutes. The cold solution is treated with Br_2 to oxidize any thiosulfate present, and the excess Br_2 removed by treatment with phenol. KI is added and the liberated I_2, a measure of the polysulfide present, is titrated with standard thiosulfate solution

Thiosulfate: The sample is treated with $BaCl_2$ and the resulting precipitate ($BaCO_3$, $BaSO_3$, and $BaSO_4$) is thrown down by centrifuging An aliquot of the clear solution is pipetted off into a buffer of about pH 7. H_2S is boiled off under vacuum. The thiosulfate present is determined by titration with iodine, after acidification with acetic acid.

Sulfite: The sample is treated with $BaCl_2$ and centrifuged. The solution is filtered and the precipitate washed free of sulfide. Sulfite in the precipitate is determined by treatment with HCl and a measured quantity of iodine solution, followed by titration of the excess iodine with thiosulfate.

Sulfate: The precipitate from the thiosulfate determination is filtered, washed, and treated with hot HCl, which dissolves the $BaSO_3$ present. The $BaSO_4$ remaining is filtered through a weighed Gooch crucible, washed, heated moderately, and weighed.

The data obtained by Theis and Ricker are shown in Table 64, and Figures 41 to 44. They reveal (1) that the sulfide content undergoes an initial rapid decrease during the first two days of treatment, longer treatment causing

only a slight further decrease in sulfide; (2) the polysulfide content increases markedly during the first 24 hours of treatment, beyond which time little change takes place; (3) the thiosulfate content increases rapidly during the first 48 hours, after which the increase is slow but positive—the curve representing thiosulfate increase is complementary to the sulfide curve; and (4) the sulfite concentration changes at practically the same rate, whether hide is present or not.

These investigators further studied the unhairing systems containing 0.2*N* sodium hydroxide only and calcium hydroxide and sodium sulfhydrate. These data are shown in Table 65 and Figures 41 to 44.

Table 64

(30 g dry hide in 300 cc 0.2*N* NaOH, with 0 5 g NaSH)

Total gram sulfur dissolved as	(Blank) 0 hours	28 hours	40 hours	78 hours	99 hours	123 hours	144 hours	(Blank) 170 hours
Sulfide	2048	.1213	.1133	.1235	1150	.1119	.1001	1740
Polysulfide	0007	0225	.0217	.0210	0203	0210	.0217	0019
Thiosulfate	0063	.1124	.1266	1313	1376	1541	.1720	.0312
Sulfite	0180	0146	0143	.0118	0116	0109	0109	0154
Sulfate	nil	0098	0065	0073	0090	.0088	0088	.0020
Total S	2374	2971	3024	3028	.3059	.3075	3100	2347
g sulfur dissolved from hide	..	0597	0650	.0654	.0685	.0701	.0726	.....
Total sulfur by addition	2298	2806	.2824	2949	.2935	3067	3135	2245
% Recovery	96 7	94.5	93 5	97.4	96 0	99 7	101 0	95 6

Table 65

(30 g dry hide in 300 cc 0.2*N* NaOH)

Total gram sulfur dissolved as	0 hours (NaOH alone)	31 hours	79 hours	127 hours	155 hours
Sulfide	. .	.0121g	.0075g	0074g	0086g
Polysulfide		.0042	.0060	.0071	.0080
Thiosulfate	.	.0265	0393	0428	.0459
Sulfite	.	.0094	.0105	.0109	.0109
Sulfate	..	.0049	0033	0064	.0064
Total S	.0008g	.0546	.0604	.0659	.0693
Sulfur dissolved from hide	. ..	.0538	.0596	.0651	0685
Total S by addition	. ...	.0571	.0666	.0746	0798

These data definitely indicate that the sulfide concentration, derived from the hair, rises and then decreases; the polysulfide increases to a more or less constant value; the thiosulfate increases steadily throughout; and the total sulfur abstracted increases continually.

The results obtained by Theis and Ricker thus indicate that the mechanism of the reaction between the various sulfides would be the action of the SH^- ion upon cystine linkages, breaking them and, perhaps, rebuilding a different type of linkage and forming also a polysulfide:

$$R—S—S—R + 2NaHS \rightarrow 2RSH + Na_2S_2$$
$$—CH_2—S—S—CH_2— \rightarrow —CH_2—S—CH_2 + S$$

The formation of polysulfides causes the unhairing solution to become

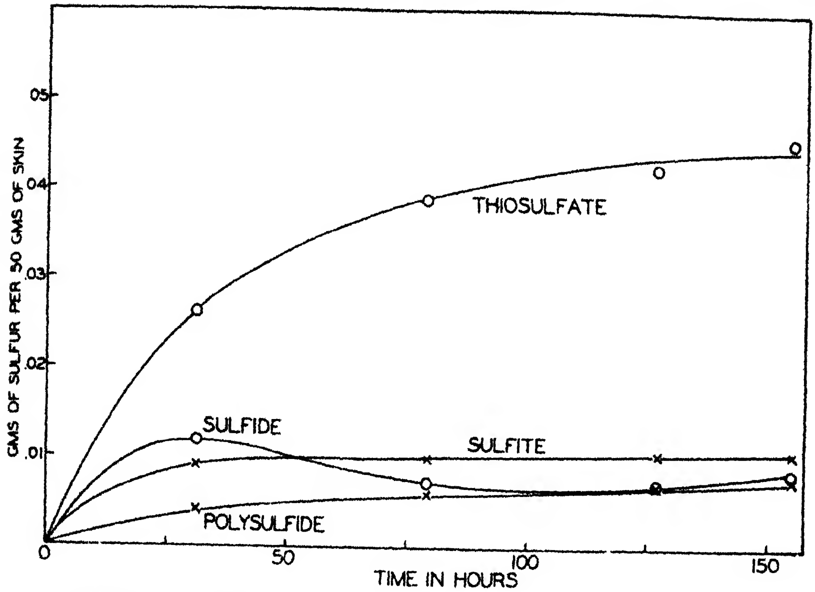


Figure 41. Showing dissolved sulfur distribution from unhairing liquor containing sodium hydroxide and no sulfur sharpener.

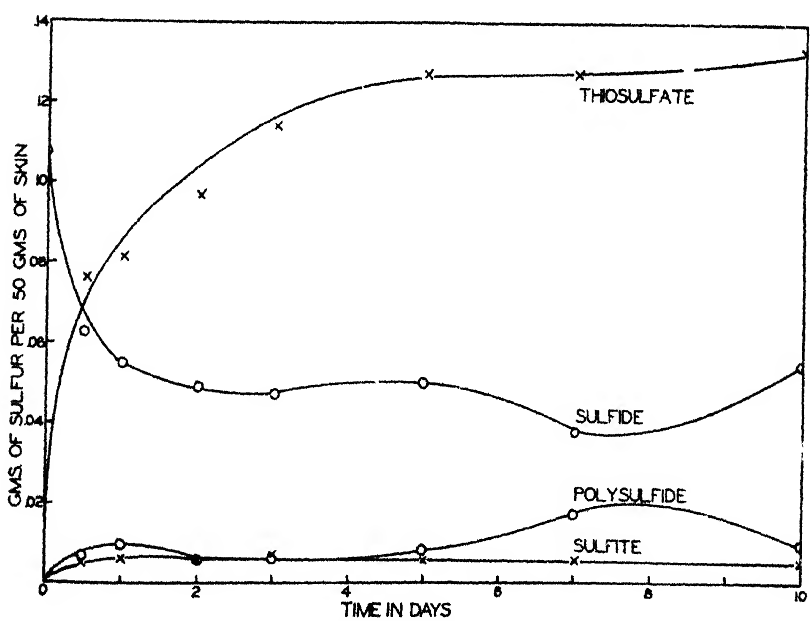


Figure 42. Showing dissolved sulfur from an unhairing solution containing lime and sodium sulfhydrate.

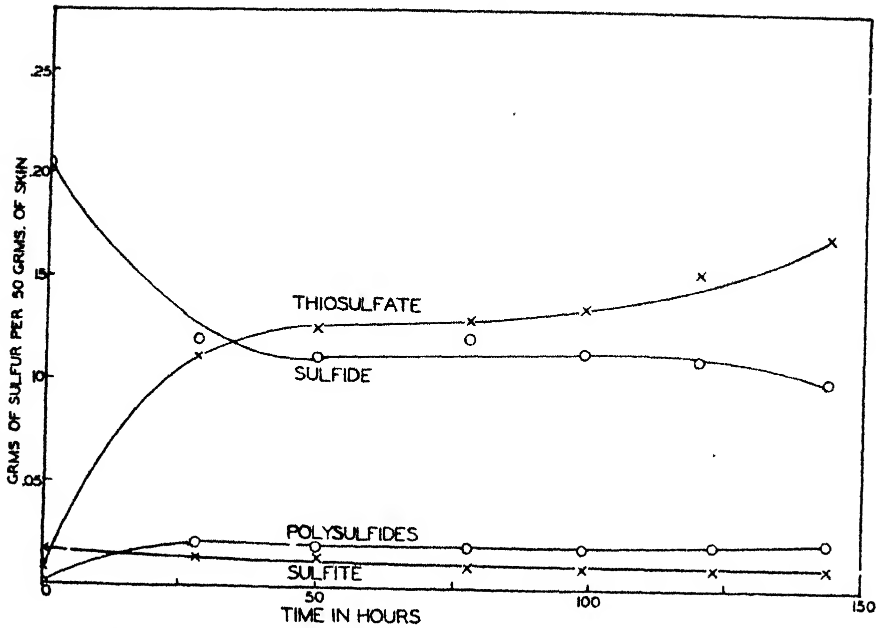


Figure 43. Showing dissolved sulfur distribution from unhairing liquor containing sodium hydroxide and sodium sulfhydrate.

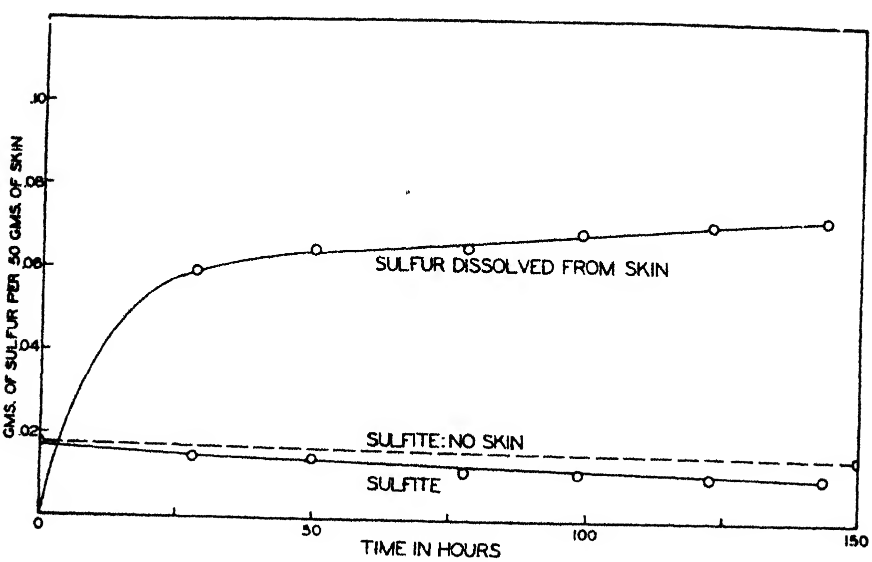


Figure 44. Showing sulfur dissolved from skin during treatment with sodium hydroxide and sodium sulfhydrate.

yellow. There is probably a reaction between sulfur or polysulfide and the sulfite formed:

$$Na_2S_2 + Na_2SO_3 \rightarrow Na_2S + Na_2S_2O_3$$
$$S + Na_2SO_3 \rightarrow Na_2S_2O_3$$

In a practical way, the suggested mechanism is borne out by the unhairing solution having a yellow color, by its low and constant values of polysulfide and sulfite ion, and by ever-increasing amounts of thiosulfate. The data shown by Theis and Ricker definitely indicate that the added sulfide or sulfhydrate is not simply and directly oxidized to thiosulfate but goes through a series of reactions before being completely oxidized to thiosulfate. These same data indicate that thiosulfate formation is important, and may be the end product of the oxidation of the sulfur compounds during depilation.

Vago,[50] in 1937, discussed the liming of skins and the immunization of hair fibers by alkali. In this work he discussed the principle of the two-bath method of liming: namely, the use of a neutral or weakly alkaline bath of hydrosulfide and then a bath containing lime or other alkali. He further points out that by washing in between these two baths, weak alkali sulfide is formed in the hair pockets, thus weakening and destroying the hair roots but not the actual hair itself. Vago also suggested that treatment by lime solution before sulfide addition immunized the hair to attack by sulfide. This fact has been recognized by the leather chemist over a long period. Vago maintains that immunization occurs in solution of pH 12.0 and thus depends upon pH value, temperature and time. It is thought that in immunization the disulfide cross-linkages are broken:

$$RCH_2-S-S-H_2CR \xrightarrow{H_2O} RCH_2-SH + RCH_2-SOH$$

The sulfenic acid, being unstable, tends to decompose: $RCH_2SOH \rightarrow H_2S + RCHO$. Thus in order to produce immunization, new cross-linkages must be formed, probably by the reaction of the sulfenic acid grouping with the amino groups of lysine and arginine

$$-CH_2SOH + H_2N- \longrightarrow -CH_2S-NH + H_2O$$

Or possibly the aldehyde groupings, produced by decomposition of the sulfenic acid, may react as follows:

$$R-CHO + H_2N-R \rightarrow R-CH=N-R + H_2O$$
$$R-CHO + HS-R \rightarrow R-CHOH-S-R$$

Although this hypothesis is consistent with the loss of half the sulfur, it fails to explain certain other observed facts when applied to alkali-treated wool keratin, namely, a weak fiber and high alkali-solubility. Thus the immunization of the hair may be brought about by the formation of the

—CH_2—S—CH_2—linkage, as postulated by Horn, Jones and Ringel. Immunization will be discussed elsewhere in this chapter.

In 1933 Moore[34] investigated the accelerating effects of various metallic sulfides on the unhairing of hides and skins. His data are shown in Tables 66 and 67. Moore's data indicate that unhairing is accelerated by the addition to lime suspensions of arsenic disulfide, arsenious sulfide, stannic sulfide, the sulfides of lithium, sodium, potassium, cesium, aluminum, magnesium, calcium, strontium, and barium.

Table 66. Some Sulfides which Accelerate the Unhairing of Steer Hide by Calcium Hydroxide Suspensions

Substance	Formula	Concentration: Millimoles per liter	Concentration: Mg per 100 cc	Apparent action on the hair
Lithium sulfide	Li_2S	6.4	29	none
		19.2	87	marked
Sodium sulfide	Na_2S	6.4	50	none
		19.2	150	marked
Potassium sulfide	K_2S	6.4	71	none
		19.2	213	marked
Cesium sulfide	Cs_2S	6 4	190	none
		19.2	570	marked
Magnesium sulfide*	MgS	6.4	36	none
		19.2	108	marked
Calcium sulfide*	CaS	6.4	46	none
		19.2	138	none
Strontium sulfide*	SrS	6.4	76	none
		19.2	228	marked
Barium sulfide*	BaS	6.4	108	none
		19.2	324	marked
Aluminum sulfide*	Al_2S_3	6.4	96	marked
		19.2	288	very marked hide stained green
Arsenic disulfide (realgar, red arsenic)	As_2S_2	6.4	137	none to marked
		19.2	411	marked to very marked
Arsenious sulfide (orpiment)	As_2S_3	6.4	158	none to moderate
		19.2	474	marked to very marked
Stannic sulfide	SnS_2	6.4	117	none, accelerated inconsistently
		19 2	351	none

* Impure preparations The concentrations given are those of the pure substance

Table 67. Some Sulfides which do not Accelerate the Unhairing of Steer Hide by Calcium Hydroxide Suspensions

(Each sulfide tested in concentrations of 6.4, 19 2 and 57.6 millimoles per liter.)

Substance	Formula	Unhairing	Stains
Cuprous sulfide	Cu_2S	slightly retarded	brown and purple in high concentrations
Cupric sulfide	CuS	slightly retarded	brown and purple in high concentrations
Silver sulfide	Ag_2S	unaffected	none
Zinc sulfide	ZnS	unaffected	none

Table 67.—*Continued*

Substance	Formula	Unhairing	Stains
Cadmium sulfide	CdS	unaffected	none
Mercuric sulfide (red)	HgS	unaffected	none
Mercuric sulfide (black)	HgS	unaffected	none
Thallous sulfide	Tl_2S	retarded 2 to 4 days	black
Stannous sulfide	SnS	unaffected	none
Lead sulfide	PbS	unaffected	black in high concentrations
Arsenic pentasulfide	As_2S_5	unaffected	none
Antimony trisulfide	Sb_2S_3	unaffected	none
Antimony pentasulfide	Sb_2S_5	unaffected	none
Bismuth sulfide	Bi_2S_3	unaffected	dark interior
Molybdenum trisulfide	MoS_3	unaffected	orange
Molybdenum pentasulfide	Mo_2S_5	unaffected	orange
Tungsten trisulfide	WS_3	unaffected	none
Manganous sulfide	MnS	unaffected	none
Manganic sulfide	MnS_2	unaffected	none
Ferrous sulfide	FeS	unaffected	none
Ferric sulfide	Fe_2S_3	unaffected	brown interior
Cobaltous sulfide	CoS	unaffected	none
Nickel sulfide	NiS	unaffected	none

Moore also investigated the effect upon unhairing of various added nitrogen compounds. In this study, he found that the following compounds accelerated unhairing: methylamine, ethylamine, dimethylamine, ethanolamine, ethylenediamine, hydroxylamine, hydrazine, guanidine tetramethyl ammonium hydroxide and piperidine.

In 1937 and 1938, Windus and Turley[52] made a comprehensive study of the unhairing effect of various mercaptans. The results of their investigation are shown in Tables 68, 69, and 70. Windus and Turley suggest that the action of the mercaptans is purely chemical, since there appears to be a definite relationship between the amount of mercaptans used and the degree of hair attack. These investigators postulate that the variation in the activity of the homologous mercaptans appears to depend upon the structure of the molecule. Table 71 indicates that the activity decreases and finally disappears according as the compound is aliphatic, alicyclic, or aromatic.

Table 68. The Unhairing Effect of Ethyl Mercaptan in the Presence of Lime (Domestic Calf skin).

Experiment	Mercaptan (%)	Strength of solution (%)	Molarity	Remarks
1	0.1	0.02	0.0032	Poor hair slip in 2 days. Good hair. Somewhat better than lime alone.
2	0.5	0.10	0.016	Good hair loosening in 2 days. No hair damage.
3	1.0	0.20	0.032	Satisfactory hair slip in 2 days. Very little hair damage.
4	1.5	0.30	0.048	Hair pulped in 4 hours. Hair becomes very loose.
5	2.0	0.40	0.064	More profound hair damage than in 4.

Table 69. Comparison of the Unhairing Action of Chemically Equivalent Quantities of the Lower Aliphatic Mercaptans (Domestic Calf skin).

Experiment	Substance	Per cent	Strength of Solution (%)	Molarity	Result
1	Ethyl Mercaptan	1.00	0.20	0.032	Fairly satisfactory hair slip in 2 days
2	Propyl »	1.25	0.25	0.032	Better hair slip than in 1.
3	Butyl »	1.45	0.29	0.032	Excellent hair slip in 2 days. Most active of series.
4	Amyl »	1.70	0.34	0.032	About same effect as in 2.

Table 70. Some Organic Thiol (—SH) Compounds which Accelerate Unhairing.

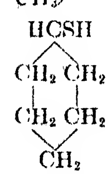
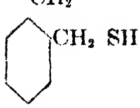
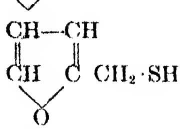
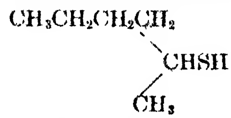
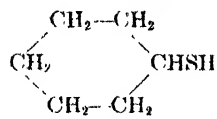
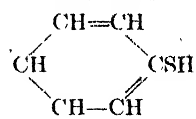
	Type	Substance	Formula
Mercaptans	Aliphatic Primary	Methyl mercaptan	CH_3SH
	Aliphatic Primary	Ethyl mercaptan	C_2H_5SH
	Aliphatic Primary	Propyl mercaptan	C_3H_7SH
	Aliphatic Primary	Butyl mercaptan	C_4H_9SH
	Aliphatic Primary	Amyl mercaptan	$C_5H_{11}SH$
	Aliphatic Primary	*n*-Hexyl mercaptan	$C_6H_{13}SH$
	Aliphatic Primary	Decyl mercaptan	$C_{10}H_{21}SH$
	Aliphatic Primary	Cetyl mercaptan	$C_{16}H_{33}SH$
	Aliphatic Primary	Dithioethylene glycol	CH_2SH | CH_2SH
	Aliphatic Secondary	*Sec.*-hexyl mercaptan	$\begin{matrix}C_4H_9\\CH_3\end{matrix}\!\!>CH\ SH$
	Aliphatic Tertiary	*Tert.*-butyl mercaptan	$\begin{matrix}CH_3\\CH_3\\CH_3\end{matrix}\!\!>C\ SH$
	Alicyclic	Cyclohexyl mercaptan	
	Aralkyl	Benzyl mercaptan	
	Heterocyclic	Furfuryl mercaptan	
	Modified Acid	Thioglycollic acid	$SH\ CH_2COOH$
	Modified Amino acid	Cysteine hydrochloride Glutathione (reduced form)	$SH\ CH_2CHNH_2COOH\ HCl$
	Modified Peptide	Butyl carbinol mercaptan	$SH\cdot CH_2CH_2OCH_2CH_2OC_4H_9$

Table 70.—*Continued*

	Type	Substance	Formula
Mercaptides	Aliphatic Primary	Sodium butyl mercaptide	C_4H_9SNa
		Calcium butyl mercaptide	$(C_4H_9S)_2Ca$
	Aralkyl	Sodium benzyl mercaptide	$C_6H_5CH_2SNa$

Table 71

n-Hexyl mercaptan	$CH_3CH_2CH_2CH_2CH_2CH_2SH$	very active
Sec.-hexyl mercaptan		very active
Cyclohexyl mercaptan		slightly active
Phenyl mercaptan		inactive

Windus and Turley state that the outstanding property of the mercaptans is their ease of oxidation to a disulfide, and that this property is responsible for the unhairing activity. These investigators further point out that all amines containing an aromatic group in the molecule are negative in their unhairing action, whereas in the case of mercaptans the thiol or SH group must be attached directly to the aromatic nucleus for the compound to be inactive. They state that the action of the aromatic mercaptans appears to be a significant exception to the Merrill-Marriott hypothesis of unhairing, and that any satisfactory mechanism of unhairing will of necessity have to take into account the action of the different unhairing reagents used today.

In 1938 Windus and Turley[52] made a quantitative study of the unhairing action of the mercaptans. For this study they used benzyl mercaptan and *p*-thiocresol. Their experimental formula was:

Calf skin	60 grams	100%
Water	300 ml	500%
Lime	6 grams	10%
or Caustic soda	1.2 grams	2%
Mercaptan	0.6 grams	1%
Temperature 70–75 F		

Since the mercaptans are easily oxidized by air in alkaline solution, a nitrogen atmosphere was used. They found that after two days, the skin treated with benzyl mercaptan in lime or caustic soda unhaired readily, with little

or no action on the hair. On the other hand, skin treated with *p*-thiocresol showed only slight hair loosening but no real hair slip. Since these investigators were interested in proving the reduction theory of unhairing, they investigated the change of the SH group to that of the S group. Their findings in this regard are shown in Table 72.

Table 72

Compound	Amount of mercaptan used (grams)	Disulfide isolated — Without skin	Disulfide isolated — With skin	% Oxidation — Without skin	% Oxidation — With skin
Benzyl mercaptan CH_2SH					
with lime (10%)	0.6	0.02	0.44	3	73
with caustic soda (4%)	1.5	0.03	1.16	2	77
with caustic soda (2%)	0.6	0.008	0.48	1	80
p-Thiocresol					
with lime (10%)	0.6	0.006	0.015	1	$2\frac{1}{2}$
with caustic soda (4%)	0.16	0.01	0.02	2	3

It is interesting to note that benzyl mercaptan is oxidized to the disulfide in the presence of skin, whereas *p*-thiocresol is not, under the given conditions.

Windus and Turley also studied the unhairing action of naphthylmethyl mercaptan (Table 73).

Table 73

	With skin — Disulfide isolated	With skin — Per cent Oxidation
Benzyl mercaptan		
with lime	0.49	81
with caustic soda	0.54	89
—Naphthylmethyl mercaptan		
with lime (2 days)	0.23	38
with lime (5 days)	0.36	60
with caustic soda (2 days)	0.27	45
with caustic soda (5 days)	0.47	78.5

Windus and Turley conclude, from their experiments, that one or more of the skin or hair proteins is responsible for the oxidation of the aliphatic mercaptan, and they suggest that the protein containing the amino acid cystine is the one involved. They picture the reaction:

$$\text{Ker—S—S—Ker} + 2C_6H_5CH_2SH \rightarrow 2\text{Ker—SH} + C_6H_5CH_2\text{—S—S}CH_2C_6H_5$$

They go on to point out that their study does not concern itself with the action of the alkali upon the various other linkages occurring in skin proteins.

The Cyanide System

In his comprehensive study of 1928, Marriott discussed the activity of sodium cyanide as an unhairing agent and showed that its reaction was

comparable to that of sodium sulfide. In 1931, Theis[41] made an extensive study dealing with sodium cyanide, and found that cyanide more than equaled the unhairing rate of corresponding concentrations of sodium sulfide. In 1941, Theis and Ricker[48] found that in contrast to the use of the sulfides, the addition of cyanides did not change materially the pH value or the dissolved lime content of the lime liquors. These data have been given elsewhere in this chapter.

There is little doubt that the alkali cyanides are excellent unhairing adjuncts when added to lime liquor. However, they are little used mainly because of their poisonous nature. Their use is confined to the production of certain types of leather or in conjunction with other unhairing agents. When they are used with alkali sulfides or arsenic trisulfide, excellent results are obtained, since the cyanide prevents the formation of polysulfides, which in turn discolor the hide and white hair This reaction is:

$$Na_2S_2 + NaCN \rightarrow Na_2S + NaCNS$$

Since polysulfides do not unhair as efficiently as do sulfides and sulfhydrates, such reagents as cyanides or sulfites act to change the former to the more active form, and thus give a more "milky" appearance to the lime liquor.

Immunization

Tannery practice has shown that if properly soaked skins are first placed in contact with a lime suspension for a few hours, the hair becomes resistant or immunized to the sulfide ion. On the other hand, if the soaked skins are pretreated with a weak sulfide solution before lime is added, the hair is much less resistant to the combined effect of lime alkalinity and sulfide ion. Hirsch[15] recently discussed the action of alkali on keratin; he stated that the alkali reacts upon the sulfide linkage, changing it to an R—S—R one, and that this type of linkage is extremely resistant to strong sulfide solutions. Hirsch calls this action of alkali upon the —S—S— linkage "immunization," and claims that it begins at pH 12.0 Vago, in 1937, had postulated the idea of immunization by lime and the formation of new cross linkages of the type:

$$CH_2SOH + H_2N\text{—} \rightarrow \text{—}CH_2\text{—}S\text{—}HN\text{—} + H_2O$$
$$R\text{—}CHO + H_2N\text{—}R \rightarrow R\text{—}CH{=}NR + H_2O$$
$$RCHO + HSR \rightarrow RCHOH\text{—}S\text{—}R$$

Theis and Blum,[42] in 1942, showed the effect of the pH value of the lime liquor upon the immunization of hair. These data will be given in Table 105. In 1943, Theis and Blum[42] studied this immunization reaction upon goat-skin hair. Their experimental procedure was the following: Goat hair was clipped from the cured skin before any soaking or liming treatment. This hair was then thoroughly washed, degreased by repeated extraction with

acetone and alcohol, and then air-dried. The hair so prepared was treated, in the right proportion of hair to solution, with the specific unhairing solutions. After treatment, the hair was quantitatively collected on a Buchner funnel, washed thoroughly, air-dried and finally dried to constant weight at 105° C. Thus loss of hair weight caused by any individual treatment might be noted. For their studies, Theis and Blum used 2 grams of degreased and dried hair, 200 ml solution, and specific time periods as noted in the tables. After the hair was removed and dried, the dried samples were placed in 200 ml of 0.1N sodium hydroxide solution maintained at 65° C for one hour. These samples were then again collected, washed and dried to constant weight. This second loss of hair weight is known as alkali-solubility.

Theis and Blum investigated the effect of lime, sulfide and cyanide pretreatment upon the hair and obtained the data shown in Tables 74, 75, 76, and 77.

Table 74. Effect of Lime Pretreatment.

Na_2S added	Final pH	Hair loss during treatment (%)	Alkali solubility treated hair (%)	Sulfur in treated hair (%)
0.00	12.58	5.37	5.0	1.68
0.05	12.60	5.83	4.6	2.03
0.13	12.60	5.70	6.6	1.98
0.25	12.62	6.43	6.6	1.96
0.50	12.72	9.70	7.8	2.16
$Ca(HS)_2$ added				
0.13	12.50	5.20	5.7	1.97
0.25	12.41	6.00	7.5	1.95
0.50	12.40	7.47	8.3	2.02

All samples pretreated with a lime suspension for 48 hours.
Na_2S added (gms/100 ml solution) to lime suspension and in contact with hair for an additional 72 hours.

Table 75 Effect of Na_2S Pretreatment

Na_2S used	Final pH	Hair loss during treatment (%)	Alkali solubility treated hair (%)	Sulfur in treated Hair (%)
0.00	12.58	6.40	4.80	1.68
0.05	12.70	. . .	6.30	2.17
0.13	12.77	7.40	9.00	2.12
0.25	12.90	59.10	100.00	. . .
0.50	12.96	100.00	. . .	.

Samples pretreated in sulfide solutions noted for 48 hours. After sulfide treatment, $Ca(OH)_2$ was added direct to sulfide solutions and treatment continued for an additional 72 hours.

Table 74 shows that moderate additions of sodium sulfide have but little effect upon hair previously treated with a lime suspension. On the other hand, hair pretreated with even comparatively small amounts of sulfide shows great loss in substance when subsequently treated with a lime suspension. This loss may amount to 50-100 per cent, as shown in Table 75.

Table 76. Effect of Lime Pretreatment.

KCN added	Final pH	Hair gain or loss during treatment (%)	Sulfur in treated hair (%)
0.00	12.58	−4.20	2.07
0.05	12.65	−3.33	1.70
0.13	12.76	−3.73	1.68
0.25	12.83	−4.80	1.67
0.50	12.85	−4.93	1.70

All samples treated with a lime suspension for 48 hours.
KCN added (gms/100 ml suspension) to lime suspension and added 48 hours and then in contact with hair for an additional 72 hours.

Table 77. Effect of Cyanide Pretreatment.

KCN added	Final pH	Hair gain or loss during treatment (%)	Sulfur in treated hair (%)
0.00	12.58	−4.20	2.07
0.05	12.65	−3.73	2.08
0.13	12.78	−8.17	1.94
0.25	12.87	−14.40	1.95
0.50	12.89	−21.00	1.56

Treated with respective cyanide solutions (gms per 100 ml solution) for 48 hours before addition of hydrated lime for additional 72 hours.

While cyanide is a considerably better unhairing agent than sulfide, it also causes much less loss in actual hair substance, as can readily be seen from Tables 76 and 77. It acts in a manner similar to sulfide with respect to the immunization reaction.

The Acid-Base Binding Capacity of Limed Skin

Previously, it has been shown that the skin proteins bind alkali during the liming operation, forming sodium and calcium proteinates in direct relation to the hydrogen-ion concentration of the unhairing bath. In Chapter 4, the acid-base binding power of collagen was discussed in some detail.

In 1941, Theis and Jacoby[43] made a preliminary study of the titration curves of limed skin and found that prolonged liming tended to shift the isoionic point of the collagen to a more acid point. In this work, however, Theis and Jacoby made use of the potentiometric method for determining the amount of acid or base fixed. Theis and Jacoby,[45] in 1942, made a more comprehensive study along these lines, making use of the pressing technique and their potassium iodide-iodate method for estimating the H^+ or OH^- bound by the protein. Their experimental procedure was as follows:

Goat skins, soaked for 2 days in cool water, were placed in a calcium hydroxide suspension for periods ranging from 1 to 15 days. At the expiration of a given period, the limed skin was removed, unhaired, washed, treated with acetic acid solution, and again washed. The washed skin was then dehydrated, using several changes of acetone and alcohol. The skin was then

cut into pieces 0.5 × 1.5 inches. These were used for the acid-base fixation curves.

One-gram pieces of the skin thus prepared were placed in acidic or basic solutions varying between pH 0.5 and 13.0. The solutions were 0.1*N* with respect to KCl and were rendered acidic or basic with HCl and NaOH, respectively. Seventy-two hours was allowed for equilibrium to establish itself. The equilibrium pH value was then measured by means of a Beckman glass electrode assembly, using the regular glass electrode for pH values from 0.5 to 9.0 and the special alkaline glass electrode from pH 9.0 to 13.0. The skin pieces were then thoroughly pressed at 5000 pounds per square inch until practically dry; they were then air-dried and ground to a fine powder for analysis. The methods for determining fixed acid or base and nitrogen have already been described in detail.

Figure 45 shows the complete titration curves for the limed goat skin-collagen. These curves may be interpreted as follows:

(1) The isoelectric point definitely shifts from pH 7.0 for the one-day liming period to pH 5.8 for the 15-day period. The greatest individual shift is from the 3- to the 5-day liming period. This shift of the isoelectric point might be accounted for by any one or a combination of three reasons: (a) an increase in strength of the active carboxyl groups without formation of additional acid groups; (b) the formation of additional acid groups by simplification of the protein-alkaline hydrolysis of the collagen; and (c) the breakdown of acid amid groups into active carboxyl groups and ammonia:

$$-CONH_2 + H_2O \rightarrow -COOH + NH_3.$$

(2) The maximum acid fixation at pH 1.0 increases for the period of liming. This increase of acid binding may be due (a) to development of additional basic groups through the previous alkaline hydrolysis, or (b) to a greater ease of hydrolysis (caused by the previous alkaline treatment) at pH values less than 3.0. Since it has been found that long contact with calcium hydroxide decreases the content of arginine, lysine and histidine, it must be therefore assumed that other basic groups are formed to more than compensate for the decrease in basic amino-acid groups.

(3) At pH values greater than 5.5 the various curves are shifted to the more acid region. There is every indication of a greater base-binding as the period of liming increases. At pH 12.5, the longer liming period has definitely increased the maximum base-fixation value. Such facts would seem to indicate (a) some protein simplification and the actual development of additional acid groups, or (b) the opening up of acid-amide groups as postulated earlier.

(4) In the pH range 6.0 to 9.5, all 6 curves show a plateau. As indicated, the base-fixation also increases with the increased lime period.

(5) Increased period of contact with the alkaline calcium hydroxide causes (a) increased acid-binding, (b) increased base-binding, and (c) a more acid isoelectric point.

(6) While these data seem of only theoretical interest, they are of practical importance to the tanner and gelatin manufacturer in that the changes caused by the liming operation must of necessity influence the subsequent, bating, pickling and tanning reactions

Since the liming process had such a pronounced effect upon the acid-base fixing power of collagen, Theis and Jacoby investigated the effect of prolonged liming of goat and calf hair. This hair, obtained by clipping the hair from the cured skins, was well washed in running water and then thoroughly extracted with acetone, alcohol and ether, after which it was air-dried. Portions of this hair were then treated with a calcium hydroxide suspension

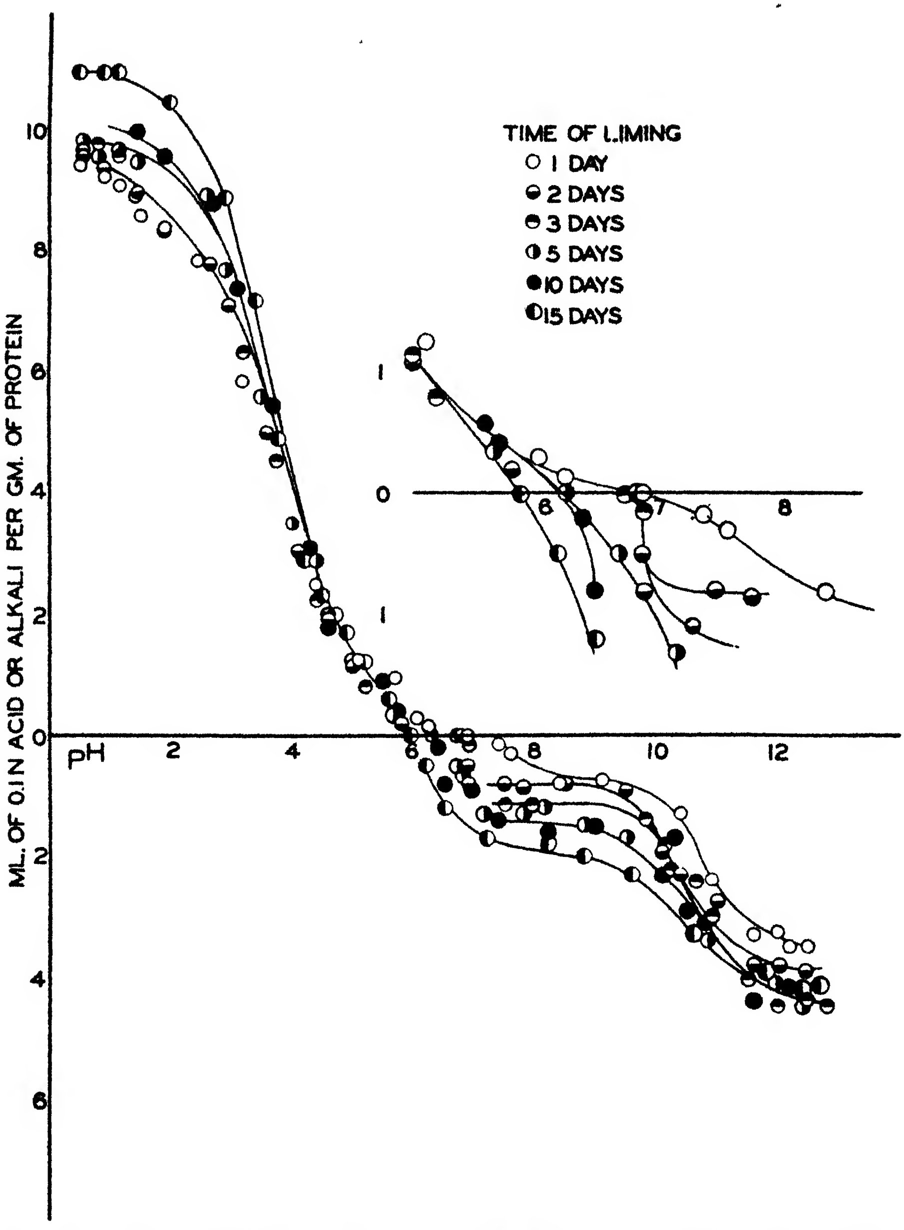


Figure 45. The effect of liming period upon acid-base binding power of animal skin collagen.

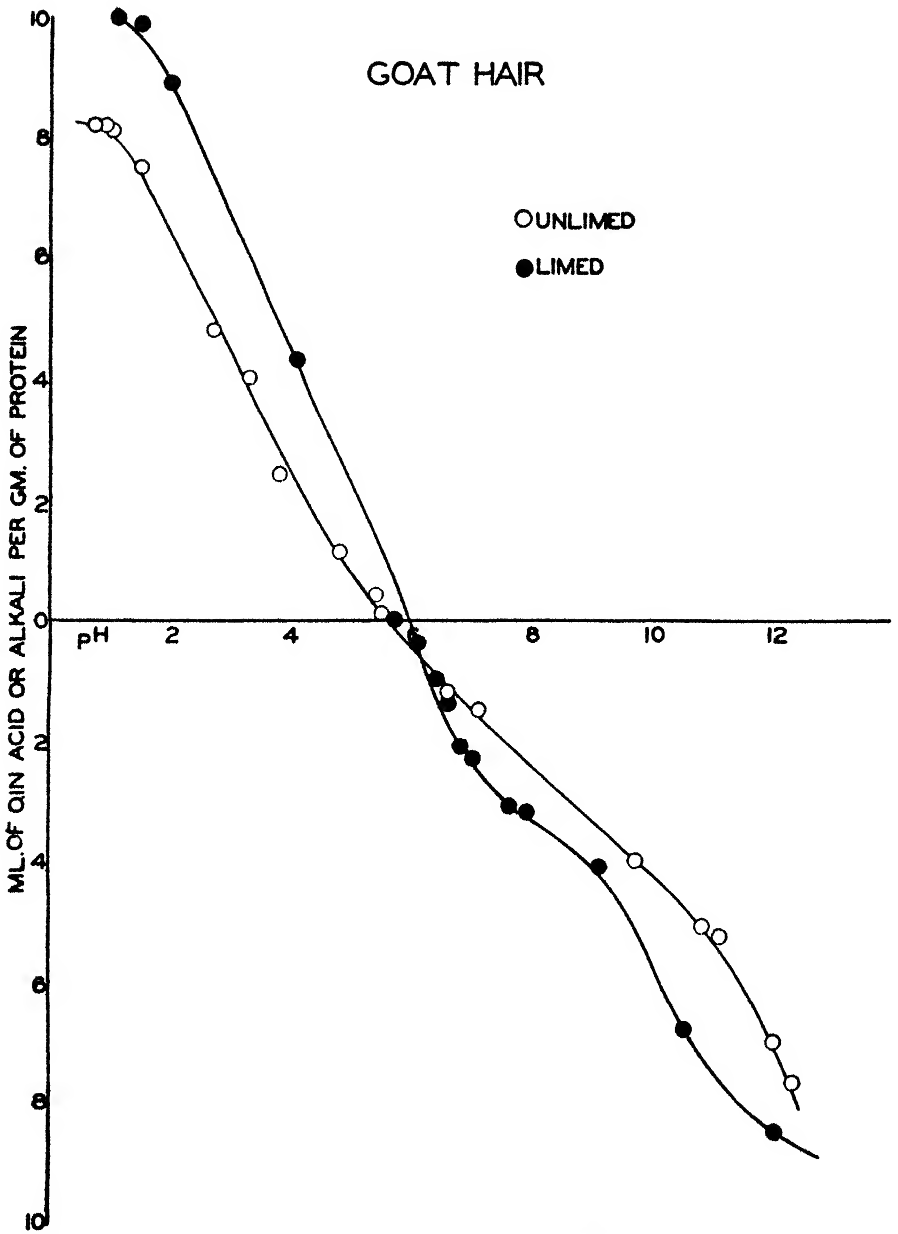


Figure 46. The acid-base binding of goat hair.

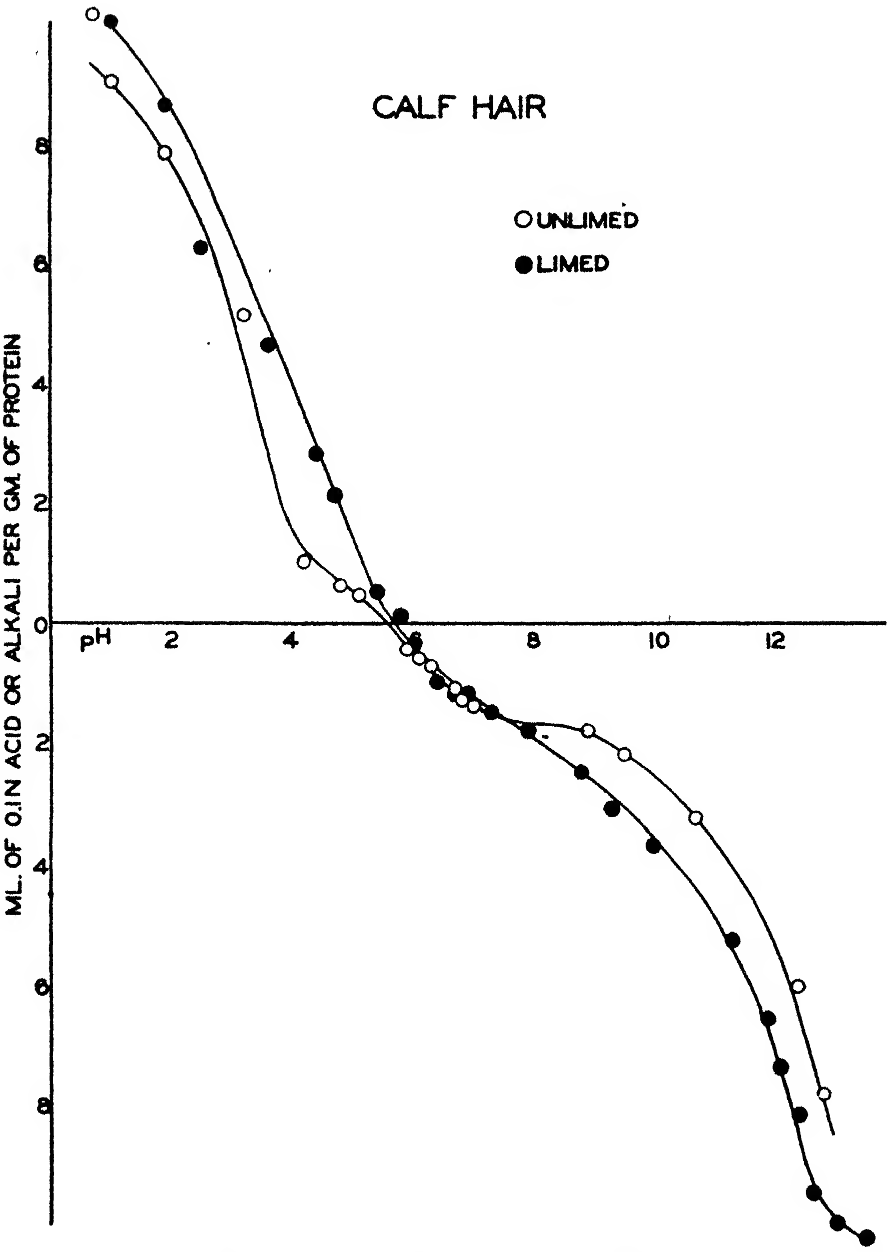


Figure 47. The acid-base binding of calf hair.

for 5 days. The treated hair was then washed, treated with dilute acetic acid, again washed, pressed, and allowed to air-dry.

One-gram portions of the treated and untreated hair were placed in acidic or basic solutions in the same manner and under the same conditions as were described for collagen. The acid or base fixed was determined in the same manner as earlier described.

Figure 46 shows the titration curves for the treated and untreated goat hair, and Figure 47 gives similar results for calf hair. The data indicate:

(1) Both the calf and goat untreated hair show an isoelectric point at 5.7. The treated hair, on the other hand, shows an approximate isoelectric point at 5.9, indicating that the alkaline treatment of the hair has shifted this point to the more alkaline region. Such a shift might indicate (a) development of stronger basic groups, or (b) activity of the sulfhydryl groups coming into being because of the reduction of the —S—S— type of protein linkage.

(2) The untreated hair of both types tends to show a maximum acid fixation of some 0.82-0.85 milliequivalent acid fixed per gram of hair substance and a maximum base binding of some 0.80 milliequivalent per gram of hair substance.

(3) The treated hair shows a greater capacity for binding both acid or base over the entire pH range studied. It is quite possible that in the alkaline treatment of the hair, additional acidic and basic groups are formed, $R—S—S—R \rightarrow R—SH + R—SOH$, and the $R—SOH$ may then change to $R—SOH \rightarrow R—CHO + H_2S$. It has been shown by Theis and Blum that hair treated with a lime suspension gives a positive test for sulfhydryl and aldehyde groups.

The liming process undoubtedly brings about very important changes in the collagen and keratin molecules, as pointed out by Wilson; the lime liquors attack and undoubtedly break up some of the cross linkages which hold the molecule backbones together. Wilson[51] has pointed out that microscopic examinations of calf skin, subjected to long liming with pure white lime, showed that though the disintegrating action is slow, there is a real and definite action, which tends to break down the protein structure. It can therefore be postulated that the liming action tends to disrupt the backbone cross linkages.

$$CO \cdot HN \rightarrow COOH + H_2N$$

liberating, and thus increasing, the number of free carboxyl and amino groups and at the same time bringing about a certain weakening of the collagen molecule itself. The titration curves shown in Figure 45 definitely indicate that both the acid- and base-binding capacity of the collagen is increased by the liming operation, and thus are in accord with the above statements.

In the case of the hair keratins, there is still an additional effect, namely the breakdown of the —S—S— linkage of cystine to a probable —SH linkage. It is well known that excess sulfides or excess caustic alkali bring about complete weakening of the keratin molecule.

The Chemical Action Due to Liming

Tanning literature abounds with the statement that one of the main functions of a lime liquor is the saponification of the hide fat into lime soap.

From a purely chemical viewpoint, this statement is rather difficult of acceptance, because lime soaps are formed slowly in the cold, and once formed, they are a dry and amorphous or sticky mass and have little or no diffusing power. McLaughlin and Theis,[28] in 1925, made a study of the action of various lime liquors upon the saponification of animal-skin fat. For this investigation, they extracted the lipid material from some ten Domestic hides. The fat so extracted was thoroughly mixed before use. Equivalents of the fat and $Ca(OH)_2$ solution were agitated twice daily at 20° C for 5 days and the saponification rates determined. Solutions of $Ca(OH)_2$ and Na_2S were also employed in another series of experiments. Their data are shown in Table 78.

Table 78

	Per cent saponification					
Hours	Straight $Ca(OH)_2$	+0.1% Na_2S	+0.2% Na_2S	+0.4% Na_2S	+0.6% Na_2S	+1.0% Na_2S
1	4.0	13.3	13.3	15.6	16.7	16.7
2	4.0	4.4	5.0	5.6	6.7	12.8
6	4.0	4.4	5.0	5.6	6.7	7.2
24	5.0	4.4	5.0	5.6	6.7	7.2
48	3.0	4.4	5.0	5.6	6.7	7.2
72	2.0	4.4	5.0	5.6	6.7	7.2
96	2.0	4.4	5.0	5.6	6.7	7.2
120	2.0	4.4	5.0	5.6	6.7	7.2
Total	26.0	41.1	48.3	54.8	63.6	72.7

These data indicate only about 26 per cent saponification in 120 hours by $Ca(OH)_2$ and some 73 per cent by strong Na_2S--$Ca(OH)_2$ solution. It further appears that mixtures of Na_2S-$Ca(OH)_2$ saponify the skin fat in some direct relation to the proportion of Na_2S present in the unhairing liquor. In 1926, McLaughlin and Theis[30] made a further study of skin-fat saponification, but in these experiments they used small hide cubes and allowed the saponification to occur within the skin structure—more nearly parallel to actual processing conditions. They determined degree of saponification by assuming that the soap so formed was no longer soluble in ordinary fat solvents. These investigators found little or no action by lime water, but if the saturated lime solution contained excess solid $Ca(OH)_2$ saponification occurred. Their data (Tables 79 to 83) indicate that there must be an excess of alkali present for saponification to occur—that is, an excess over that necessary to satisfy the combining capacity of the protein itself. It is also apparent that weak sodium hydroxide possesses no greater action on the fat of the skin than does ordinary $Ca(OH)_2$, despite its well known power of more rapid action on the extracted fat. This phenomenon may be associated with the fact that its swelling action on the skin proteins is much greater than that of lime and the compressed condition of the swollen skin retards its saponifying action.

Table 79

Specimen number	1		2		3		4		5	
	% Fat left	Saponification	% Fat left	Saponification	% Fat left	Saponification	% Fat left	Saponification	% Fat left	Saponification
Control	0.784		1.382		0.784		1.054		1.856	
After 1 day liming	0.552	30					0.892	15	1.732	7
After 2 days liming	0.510	35								
After 3 days liming					0.474	40				
After 4 days liming	0.510	35								
After 5 days liming	0.506	35								
After 6 days liming			0.936	32	0.394	50				
After 14 days liming	0.420	46			0.360	54				
After 24 days liming	0.390	50								
After 70 days liming					0.236	70				

Table 80

Treatment	% Fat left in treated hide	% Saponification
Control	2.10	
$N/100$ NaOH, not renewed	2.02	4
$N/100$ NaOH, renewed	1.91	9
$N/20$ NaOH, not renewed	1.93	8
$N/20$ NaOH, renewed	1.58	25

Table 81

Days soaked	1		2		6	
	% Fat left in treated hide	% Saponification	% Fat left in treated hide	% Saponification	% Fat left in treated hide	% Saponification
Control (no treatment)	1.86		2.09		1.38	
$N/100$ NaOH	2.15	..	2.02	3	1.35	2
$N/20$ NaOH	1.92	..	1.93	8	1.12	19
$N/10$ NaOH	1.97	..	1.58	25	1.11	20
$N/2$ NaOH	1.21	35	1.19	43	digested	..
Sat. $Ca(OH)_2+6\%$ excess	1.73	7			0.94	32

Table 82

Treatment	% Fat left in limed hide	% Saponification
Control (no treatment)	2.03	
Lime solution not renewed during 5 days	1.82	10.50
Lime solution (without excess lime) renewed each day for 5 days	1.53	25.00
Lime solution not renewed during 5 days but containing 6% excess, solid $Ca(OH)_2$	1.31	35.00

Table 83

Treatment	% Fat left in limed hide	% Saponification
Control (no treatment)	2.03	00.00
$Ca(OH)_2$ (no excess) 24 hours	2.03	00.00
$Ca(OH)_2+6\%$ excess 24 hours	1.69	17.00

Koppenhoefer[17] later made a comprehensive investigation of the lipids of various types of skin and in this study investigated the effect of various unhairing liquors on the lipids of steer hide. For the work in hand, Koppenhoefer used a heavy Colorado steer hide which had been cured for about 40 days. The hair was clipped short and cut into 3-inch strips. These strips were soaked in distilled water for 24 hours at 20° C and then placed in the respective unhairing solution for the prescribed liming period. The strips were then unhaired and the subcutaneous fat and flesh tissue removed. Koppenhoefer separated the grain from the corium and thus investigated the lipid content of both layers.

The first investigation dealt with plain liming, *i.e.*, no unhairing aid was used. Regarding this study, Koppenhoefer points out the following pertinent facts: (a) no change in the quantity of total corium lipid resulted during liming with straight lime; (b) liming does not effect either the saponification or the removal of the corium triglycerides; (c) liming causes complete neturalization of the free fatty acids of both the corium and epidermal layers; (d) the phospholipid fraction is completely saponified; (e) calcium soaps are formed during straight liming.

In his study of the effect of sulfide-lime liquors on hide lipids, Koppenhoefer obtained very interesting data, in good agreement with the earlier work of McLaughlin and Theis. The data obtained by Koppenhoefer are shown in the following tables and figures.

Table 84. Summary of Lipid Distribution: Corium Lipids.

	Cured hide	Limed hide
Dry, ash-free weight (gm)	1408.	1600.
Weight lipid (gm)	41 73	41.88
Per cent of dry weight	2.96	2.62
Lipid phosphorus (mg/kg dry corium)	34.5	9.06
Cholesterol (gm/kg dry corium)	0.76	0.78
Free fatty acid (gm/kg dry corium)	1.87	0.09
Soap (as fatty acid) (gm/kg dry corium)	0.0	3.15
Triglyceride (gm/kg dry corium)	25.2	19.8

Table 85. Summary of Lipid Distribution: Epidermal Lipids.

		Limed	
	Cured	Based on cured weight	Based on limed weight
Dry, ash-free weight (gm)*	401.	455 *	278.
Weight lipid (gm)	26.9	9.12	9 12
Per cent of dry weight	6.71	2.01	3.29
Lipid phosphorus (mg/kg dry material)	186.5	17.2	28.1
Cholesterol (gm/kg dry material)	8.11	3.58	5.86
Cholesterol (as ester) (gm/kg dry material)	3.86	0.95	1.55
Free fatty acid (gm/kg dry material)	8 86	0 07	0 11
Soap (as fatty acid) (gm/kg dry material)	0.0	5 67	9.28
Wax (gm/kg dry material)	25.7	1.58	2.60

* For comparative purposes, this dry, ash-free weight of epidermal material was calculated from the weight of limed corium obtained. This was accomplished by multiplying the limed weight of corium by the ratio of epidermal to corium weights obtained by actual separation of the cured hide into these divisions.

Table 86. Summary of Lipid Distribution: Scud Lipids.

	Limed	
	Based on cured weight	Based on limed weight
Dry, ash-free weight (gm)	455.	171.
Weight lipid (gm)	16.55	16.55
Per cent of dry weight	3.64	9.68
Lipid phosphorus (mg/kg dry material)	0.0	0.0
Cholesterol (gm/kg dry material)	4.05	10.76
Cholesterol (as ester) (gm/kg dry material)	2.20	5.85
Free fatty acid (gm/kg dry material)	1.03	2.75
Soap (as acid) (gm/kg dry material)	10.87	28.9
Wax (gm/kg dry material)	6.28	16.7

Table 87. Effect of Sodium Sulfide Concentration on the Saponification of Corium Triglycerides During the Liming of Heavy Steer Hides. (Liming for 6 days at 20° C)

Gms Na_2S per 100 gms of fresh steer hide	Mg soap (as stearic acid) formed in corium per 100 grams fresh steer hide	% of total saponifiable corium lipid actually saponified
Hide A: Average lipid content, 1.14% of dry corium weight.		
0.0	27.4	13.7
0.2	35.7	13.5
0.6	24.2	18.7
1.0	36.8	22.4
1.6	74.8	38 3
2.4	89.0	50.2
3.6	...	48.7
4.8	186.	56.8
8.0	252.	79.9
12.0	259.	88.0
Hide B: Average lipid content, 2.76% of dry corium weight.		
0.0	75.1	11.8
0.2	78.5	11.8
0.6	73.1	13.8
1.0	65.6	14.3
1.6	94.4	15.6
2.4	171.	20.1
3.6	200.	19.5
4.8	138.	31.2
8.0	348.	46.7
12.0	442.	76.9
Hide C: Average lipid content, 8 42% of dry corium weight.		
0.0	45.4	2.10
0.2	110.	2.41
0.4	145.	1 90
0.6	140.	2.47
1.0	189.	6.72
1.6	213.	8.52

Table 88. Effect of Time on the Saponification of Corium Triglycerides during the Liming of Heavy Steer Hides. (Liming at 20° C)

Period of liming (days)	Mg soap (as stearic acid) formed in corium per 100 gm fresh hide	% of total saponifiable corium lipid actually saponified
A: Liming in straight lime. Average lipid content of hide, 4.73%.		
1	40.0	3.33
2	46.0	4.46
4	60.4	3.65
6	53.8	3.37
9	46.5	3.58
16	55.1	...

Table 88—*Continued*

Period of liming (days)	Mg soap (as stearic acid) formed in corium per 100 gm fresh hide	% of total saponifiable corium lipid actually saponified
B: Liming in 1.0% sodium sulfide (on fresh hide weight) lime liquors. Average lipid content of hide, 2.35%.		
1	56.2	9.06
2	81.2	15.7
4	85.5	10.8
6	...	14.3
11	105.	17.8
14	118.	18.9
C: Liming in 3.6% sodium sulfide (on fresh hide weight) lime liquors. Average lipid content of hide, 2.44%.		
1	77.7	14.2
2	100.6	17.8
4	151.	26.2
6	200.	24.6
11	223.	47.3
14	212.	31.7

Table 89. Effect of Temperature of Liming upon Saponification of Corium Triglycerides During the Liming of Heavy Steer Hides. (Liming for 7 Days.)

Temperature of liming (° C)	Mg soap (as stearic acid) formed in corium per 100 gms fresh hide	% of total saponifiable corium lipid actually saponified
A: Liming in straight lime liquors, lipid content, 8.36%		
5	36.8	0.90
10	37.4	1.88
20	45.4	2.10
30	55.5	4.06
B: Liming in 0.6% sodium sulfide (on fresh hide weight) lime liquors. Average lipid content of hide, 6.13%.		
5	78.5	...
10	74.1	4.46
20	111.	5.38
30	116.7	5.68
C: Liming in 1% sodium sulfide (on fresh hide weight) lime liquors. Average lipid content of hide, 2.76%.		
5	95.6	22.6
10	125.	22.7
20	158.	21.9
30	...	38.4

Koppenhoefer points out, from this extended investigation of the various sulfide liming processes upon the lipids of steer hide, the following: (a) saponification of the corium lipids increases but slowly with sulfide concentration, becoming appreciable at very high concentration; (b) the amount of saponification of the corium lipids increases with lipid concentration, but the actual percentage saponification actually decreases; and (c) the amount of saponification increases with time and moderate increase in temperature has but little effect.

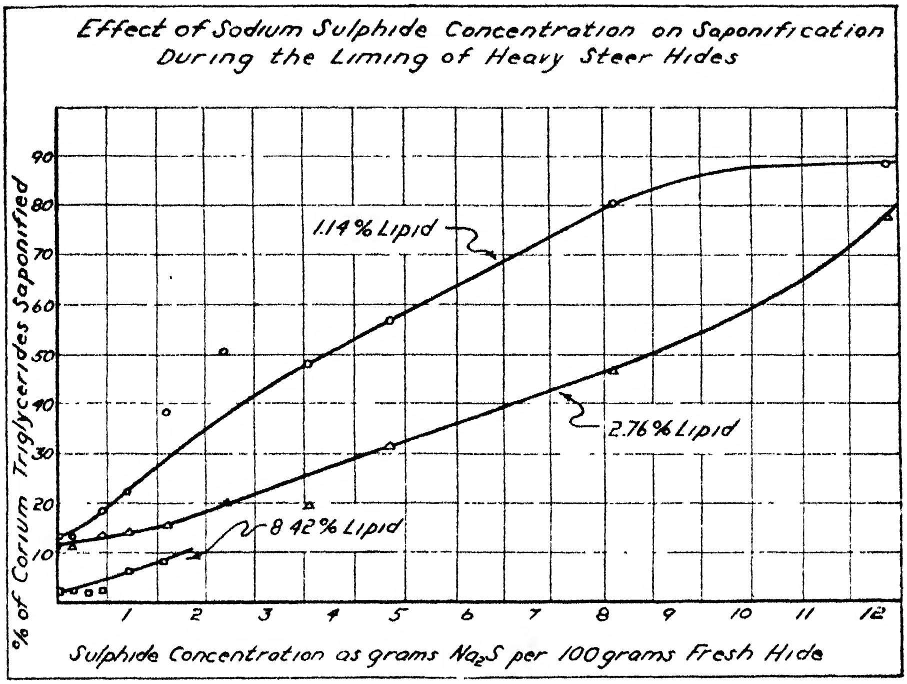


Figure 48

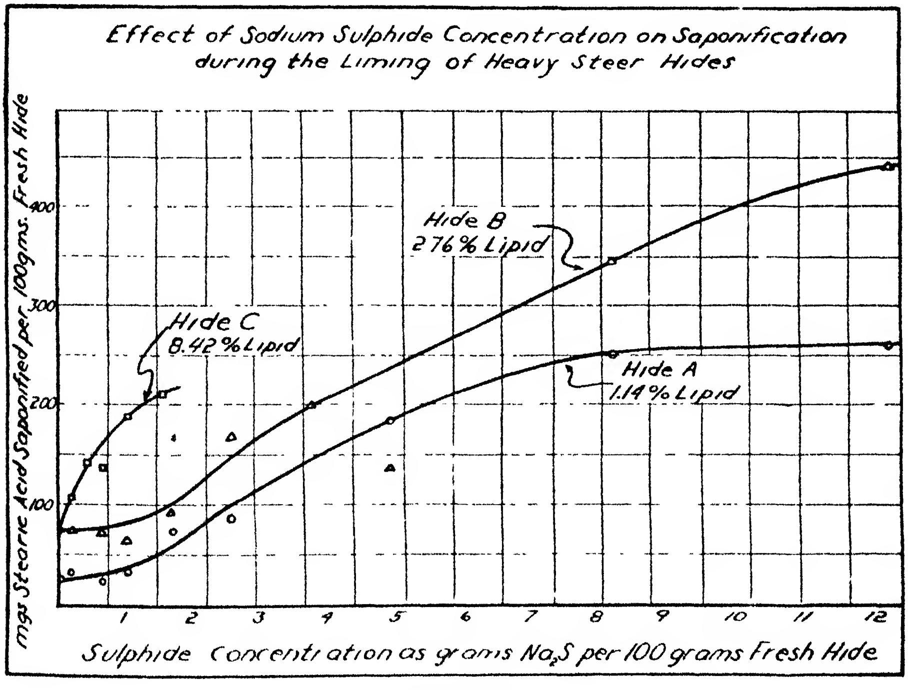


Figure 49

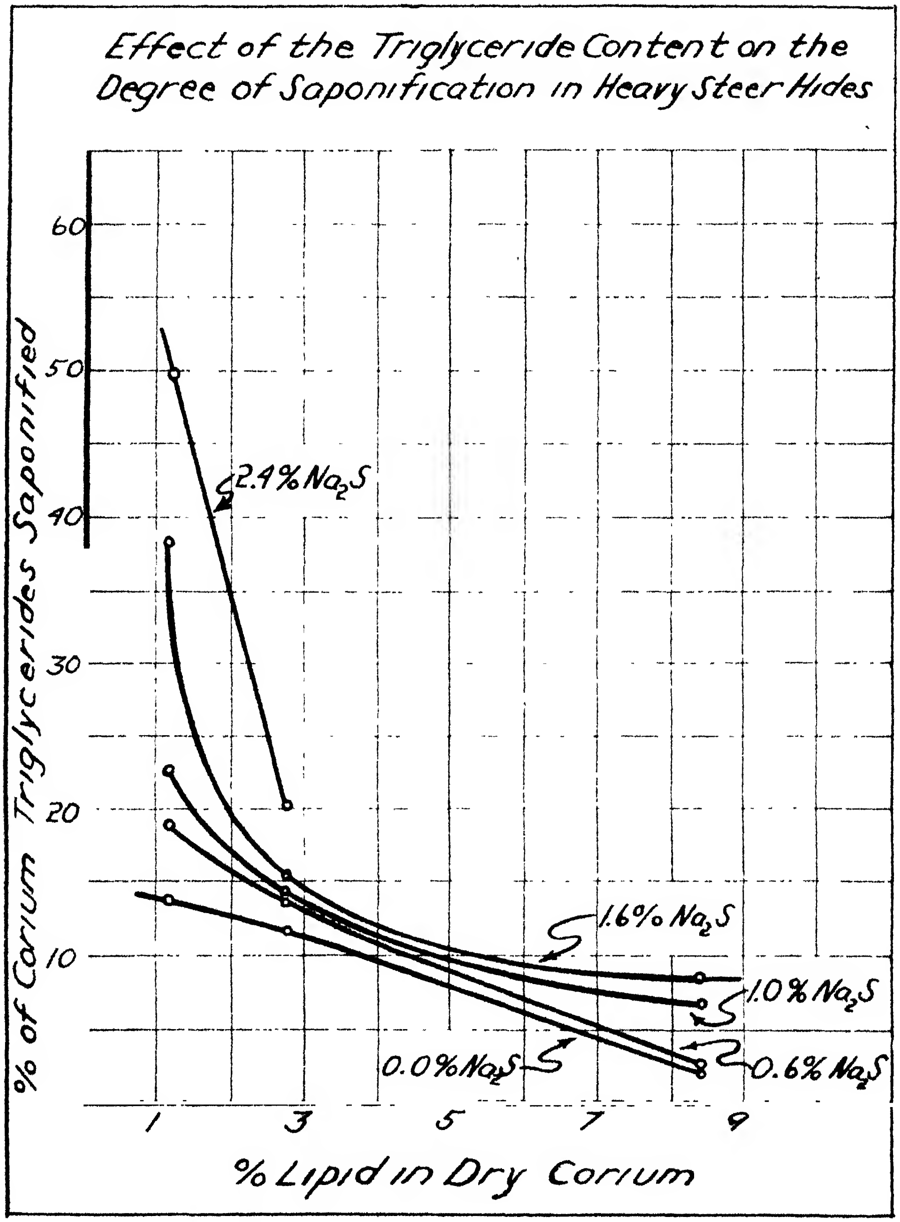

Effect of the Triglyceride Content on the Degree of Saponification in Heavy Steer Hides
% of Corium Triglycerides Saponified
60
50
40
30
20
10
2.4% Na_2S
1.6% Na_2S
1.0% Na_2S
0.6% Na_2S
0.0% Na_2S
1
3
5
7
9
% Lipid in Dry Corium

Figure 50

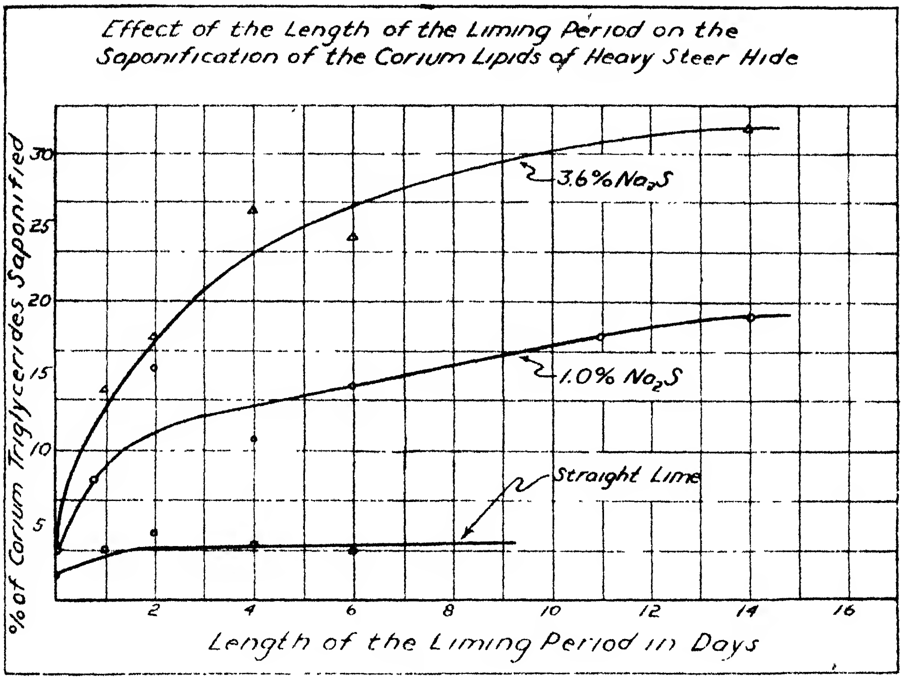


Figure 51

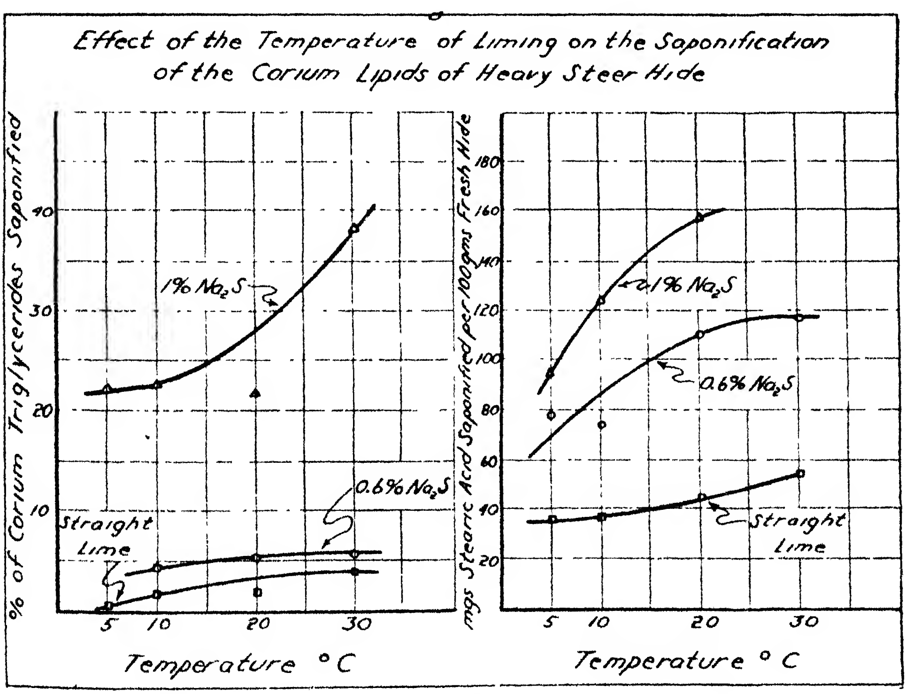


Figure 52

In his study of the effect of Pullery processes on the lipids of sheep skins, Koppenhoefer gives the data as shown in Tables 90 and 91.

Table 90. Removal of Lipid from the Sheep Skin During Various Pre-Tanning Procedures.

Procedure	Weight of Lipid (gm)	% of Total
Lipid in cured skin	113.1	100 0
Lipid recovered from soak waters	11.6	10.2
Lipid removed during wool pulling	38.1	33.7
Lipid removed from paddle solutions	5.3	4.77
Lipid recovered from lime liquor	1.5	1.33
Lipid remaining in limed skin	56.6	50.0

Table 91. Analyses of Sheep Skin Lipids.

Summary of the Lipid Distribution in the Corium Division

Condition of Skin	Fresh	Cured	Sulfided	Cured	Limed
Dry, lipid-free, ash-free weight (gm)	93.7	61.2	76.4	176.	142.
Weight of lipid (gm)	29.9	28.9	45.3	106.2	103.2
% Lipid on dry weight	31.8	47.5	59.3	60.4	72.6
Lipid phosphorus (mg/kg dry material)	393.	320.	9.8	162.	0.0
Total cholesterol (gm/kg dry material)	5.56	6.21	7.32	4.72	4.83
Triglyceride (gm/kg dry material)	194.3	403.	462.	568.	658.
Free fatty acid (gm/kg dry material)	7.52	19.7	11.5	3.81	1.41
Soap (as fatty acid) (gm/kg dry material)	...	...	35.7	.	13.8
Ash (%)	..	27.2	4.27	22.3	8.28

Koppenhoefer noted that the sulfiding of the sheep skin almost completely removed the phospholipid, and that the cholesterol remained essentially unchanged. This sulfiding treatment resulted in only a slight saponification of the corium triglycerides and only a partial neutralization of the free fatty acids.

Action on the Proteins

In addition to their action on the lipids of the hide or skin, the unhairing liquors have a distinct and positive action on its protein constituents. It has long been known that prolonged liming removes the mucoid fraction, but until recently little was known relative to the action of limes upon the proteins themselves. In 1941, Theis and Jacoby[44] investigated the action of $Ca(OH)_2$ solutions on the basic amino acid content of collagen. For this work, special hide powders were prepared. Steer hide was soaked in cold water for 24 hours and then limed in a straight lime liquor for periods ranging from 24 to 360 hours. After this period, the limed hide was removed, unhaired and carefully fleshed. The white hide was then delimed with an acetic acid solution, washed and then thoroughly dehydrated in several changes of acetone. After dehydration, the hide was ground in a Wiley

mill. This dehydrated material was then analyzed. The data relative to the basic amino-acid content are given in Table 92 and Figure 53.

Table 92

Liming Period (hours)	% Arginine	% Lysine	% Histidine	Molecular Ratio Histidine: Lysine: Arginine
24	7.75	3.64	0.43	1:9:16
72	7.14	2.50	0.34	1:8:19
120	7.14	2.50	0.34	1:7:19
240	7.14	2.28	0.33	1:12:43
360	6.52	1.50	0.12	1:13:48

A careful study of these data shows that all three of the basic amino acids, *i.e.*, arginine, lysine and histidine, are decreased by prolonged liming treatment. It appears, however, that lysine and histidine are more drastically affected than arginine. It further appears that the decrease in basic amino acid content is proportional to the liming period. The recent observation of the change in isoelectric point of collagen, from approximately pH 8 to pH 5.0, on treatment with a strong alkali, causes considerable speculation regarding the structural changes taking place within the skin during liming. Highberger and Stecker[14] state that such a shift of the isoelectric point toward a lower pH value is probably due to a structural change involving either the production of new or stronger acidic groups or some destruction of the existing basic groups. Beek and Sookne[3] had previously suggested that the many acid amide groups of collagen are broken down by the alkaline liming treatment, with formation of active carboxyl groups and free ammonia. Braybrooks[6] maintains there are some 29 of these acid amide groups per molecule of collagen, and the existence of such groups is widely accepted. The hydrolysis of such groups would be in line with the suggestion of Highberger and Stecker, since the carboxyl groups thus formed would be highly acidic. These investigators point out that the shift of the isoelectric point to a lower pH may be due to a breakdown of the strongly basic guanidino groups of the arginine residues of the collagen molecule. This particular reaction:

$$\begin{matrix}\mathrm{CO} \\ \mathrm{HC{-}(CH_2)_3NHC(NH)NH_2} \\ \mathrm{NH}\end{matrix} \xrightarrow{H_2O} \begin{matrix}\mathrm{CO} \\ \mathrm{HC{-}(CH_2)_3NH_2} \\ \mathrm{NH}\end{matrix} + \mathrm{CO(NH_2)_2}$$

is known to occur in the action of warm alkali on the free amino acid, but some doubt exists as to just how far this reaction occurs in cold lime solution. Undoubtedly the reaction takes place to a very limited extent in the cold.

Highberger and Stecker investigated the amounts of urea and ammonia formed by the action of various lime liquors on collagen. Experimentally, they placed 1.0-gram portions of collagen in 100-ml quantities of the appropriate lime liquor and allowed the reaction to proceed at room temperature.

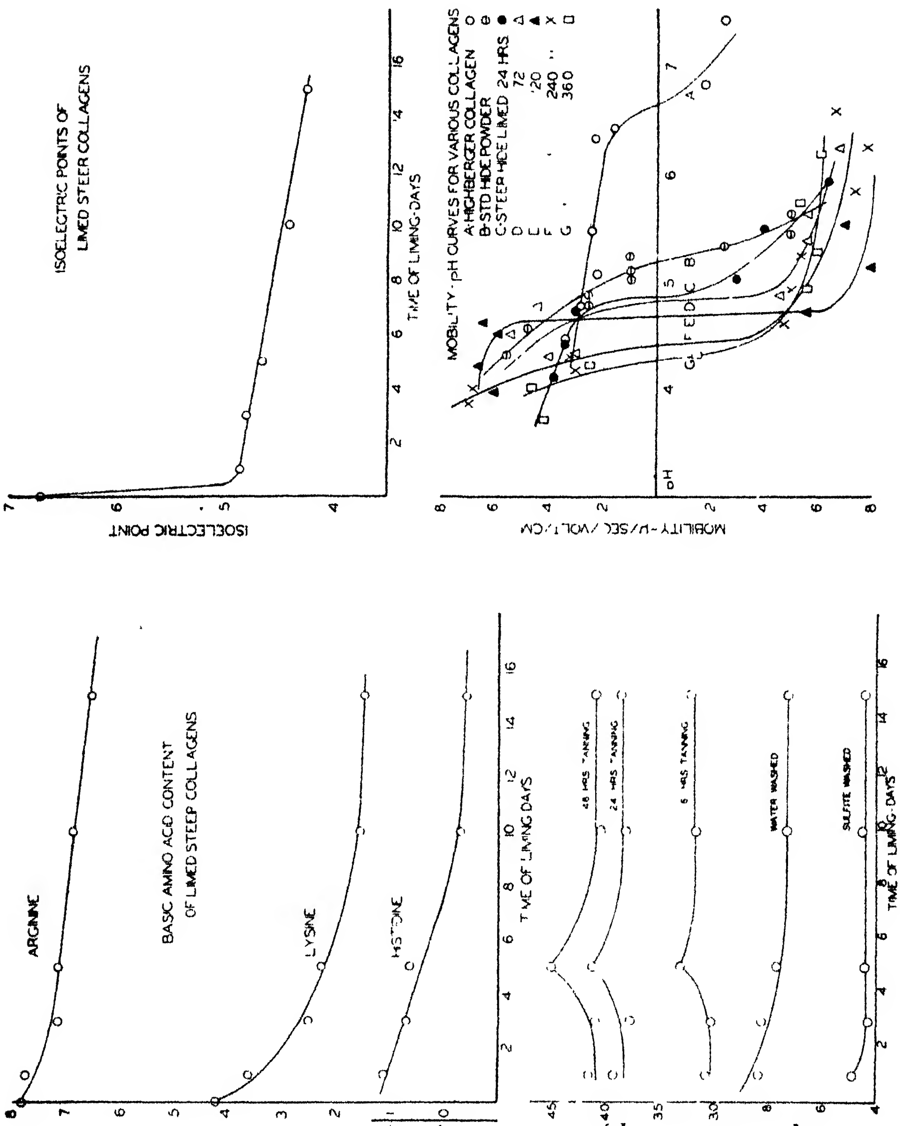


Figure 53. The Basic Amino Acid Content Formaldehyde Fixation, Cr_2O_3 Fixation, and Isoelectric Points of the 5 Special Limed Hide Powders.

The amounts of ammonia and urea were then determined by special methods. Their data are shown in Figures 54 and 55. These data definitely indicate that only a very small amount of urea, 1.0 milligram per gram collagen, is formed in a straight lime over a period of 9 days. However, with increasing causticity and with a lengthened period of liming, this value may reach 5.5 milligrams. Data given in Figure 55 however suggest that a much greater amount of ammonia is formed. Highberger and Stecker state that it appears

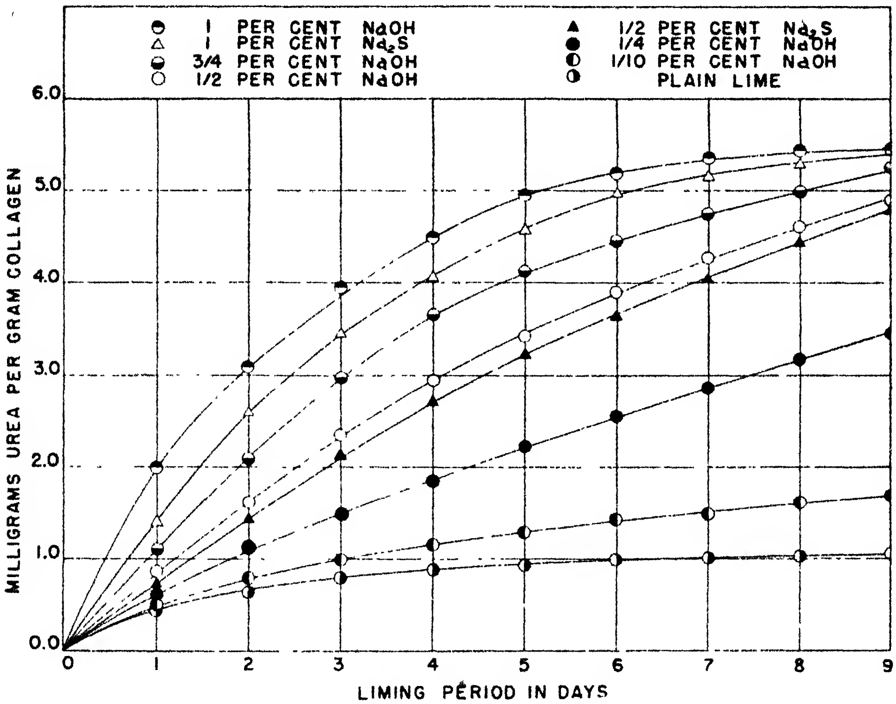


Figure 54

probable that all the acid amide groups have been hydrolyzed. They point out that their results indicate that the shift in the isoelectric point is mainly due to the hydrolysis of the acid amide groups, with subsequent formation of very acidic carboxyl groups. The data of Theis and Jacoby show that about 16 per cent of the arginine is destroyed in the interval between the 24-hour and the 360-hour liming periods. On the other hand, their data show that some 58 per cent of the lysine is destroyed. The maximum acid-binding capacity of limed skin increases with increase in the liming period, indicative of the formation of additional acid-binding groups.

Such data would suggest the formation of additional free amino groups in either or both ways (a) the possible breakdown of the polypeptide chain, giving both additional basic and acidic groups, or (b) the breakdown of certain of the imino-carboxyl linkages: CO—NH → COOH + H_2N. Such reactions would account for the additional acid- and base-binding capacity of the limed skin, but would not account for the shift in isoelectric point of the collagen. This shift might occur, however, because of the generation of the very acidic carboxyl groups from the acid amide groups destroyed by

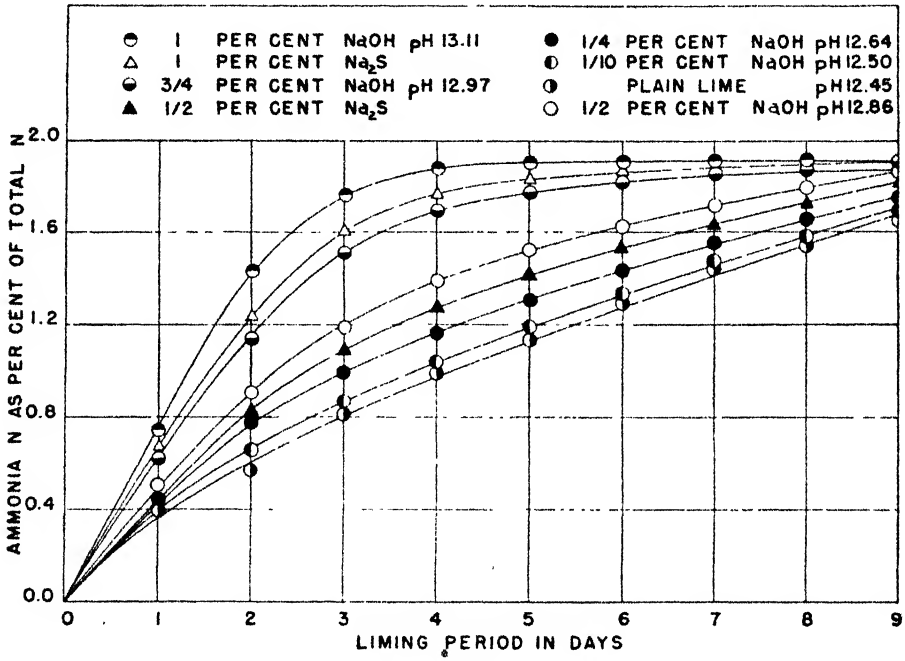


Figure 55

the hydrolysis taking place during a liming process. These additional acidic groups would not affect the base-binding capacity of the proteins, since they would in all probability be hydrolyzed. There is still a great need for further investigation along the lines started by Highberger and Stecker.

Work carried out by Theis and Steinhardt[49] indicated that certain breakdown in the collagen structure occurred during the liming operation. In further work, Theis and Blum[49] determined the shrinkage temperature of goat skin limed in six different lime liquors and over a period varying from 1 to 15 days. These data are shown in Table 93.

Table 93

Liming period (days)	Shrinkage Temperature (° C) 1	2	3	5	10	15
Straight lime*	63.1	64.3	63.5	62.3	58.9	57.3
Lime +3% Na_2S	60.2	60.8	..	60.6	58.5	55.3
Lime +0.8% NaHS	63.3	63.4	64.5	61 6	58.9	56.3
Lime +1% As_2S_3	64.3	.	67.3	63.3	59.8	59.0
Lime +1% $(CH_3)_2NH$	61.5	62.3	65.3	61.0	60.3	57.0
Lime +6% Na_2SO_3	58.5	59.0	59.3	58.5	56.0	56.3

* 10% solid $Ca(OH)_2$. Ratio, hide to liquor, 1 to 4.

These data show that sodium sulfide and sodium sulfite have the most drastic effect upon the shrinkage temperature of the limed skin. In all cases, the decrease in structural cohesion forces of the skin is most noticeable after a 5-day liming period. With the exception of the arsenic sulfide and sodium sulfhydrate, all the unhairing adjuncts listed in Table 93 increase the pH value of the lime liquor. Thus it can be stated that the straight lime, the sulfhydrate, and arsenic sulfide lime give the best results as far as the effect upon the internal structural cohesive forces is concerned. These factors have been discussed in detail elsewhere.

The effects of the specific lime liquor upon the protein constituents of the hide or skin are of a very positive nature and should show some characteristic effects upon the subsequent processes—bating, pickling, and tanning. So far as the authors are aware, no data exist in the available literature relative to such effects upon bating or pickling, but there are some data relative to aldehyde tannage. Theis and Jacoby made a preliminary investigation along such lines and found that prolonged liming decreases the amount of formaldehyde fixed. Later, Theis and Steinhardt[49] studied the aldehyde fixation of some 36 different specimens of formaldehyde-tanned leather and found that the liming period definitely affected the shrinkage temperature of the finished leather and the formaldehyde fixation. These data are shown in Table 94. Thus it can readily be noted that with increasing liming period, the structural cohesive forces are weakened and, in general, the amount of formaldehyde fixed decreases. This table also indicates that skin limed

Table 94

	Liming period (days) 1		2		3		5		10		15	
	S.T.*	CH_2O†	S.T	CH_2O	S.T.	CH_2O	S.T	CH_2O	S.T.	CH_2O	S.T	CH_2O
Straight lime	90	1.33	90	1.39	90	1.37	89	1.20	86	1.36	85	1.14
Lime + Na_2S	89	1.05	88	1.01	88	1.25	87	1.06	85	1.01	82	.91
Lime + NaHS	91	1.00	89	.97	91	1.05	88	.77	86	96	83	.81
Lime + As_2S_3	89	.93	89	1.00	90	.98	89	.80	88	.82	86	.80

* S.T. = Shrinkage temperature (° C).
† Per cent formaldehyde fixed at pH 8.

with a straight lime liquor is able to combine with more formaldehyde than skin treated with other types of unhairing solutions. Theis and Steinhardt studied the chrome tanning of skin limed in various ways and for different periods, but were able to find little or no difference in Cr_2O_3 fixation or in the shrinkage temperature of the leather so produced.

The Effect of pH Value of Unhairing Solutions

Until 1940, no accurate pH values of unhairing solutions had been determined, with the exception of solutions of calcium hydrate alone. The hydrogen electrode, while accurate for pure lime water, was not accurate in the presence of sulfides.

In 1933, Atkin, Goldman, and Thompson[2] studied the determination of pH values of lime liquors. In this work, they pointed out the inadequacy of the hydrogen, quinhydrone and glass electrodes for this important determination. They showed that since sodium sulfide hydrolyzes freely into sodium hydrosulfide and sodium hydroxide, it is rather obvious that the addition of sodium sulfide to a lime solution is equivalent to the addition of sodium hydroxide in approximately half the quantity. Thus it is apparent that the alkali must increase, and if $(Ca^{++}) \times (OH^-)^2 = K$, then the calcium ion must decrease. From this relation, Atkin, Goldman and Thompson worked out a mathematical expression

$$\text{pH} = 11.65 + \tfrac{1}{2}\,p\text{Ca}$$

for calculating the pH value of lime solutions containing sulfide ion.

In 1938, Fritsch[8] attempted to use the glass electrode and came to the conclusion that the expression worked out by Atkin, Goldman, and Thompson was best suited for this purpose.

Since 1938, a Beckman glass electrode made of special glass has been designed and built. This electrode has reasonable accuracy up to and including pH 13.5, is not affected by sulfide or sulfhydrate ions, and measures readily the pH value of all unhairing solutions encountered by the leather chemist. Theis and Ricker[46] used this electrode for the determination of hundreds of pH values of lime liquors, containing OH^-, $S^=$, HS^-, CN^-, $S_2O_3^=$, $SO_3^=$, and other ions often present in unhairing solutions. They found the electrode to function rapidly and accurately. Theis and Ricker measured the pH value of various unhairing solutions made up in such a way as to represent actual tanning conditions; (a) volume ratio liquor to skin, 4:1; (b) 10 per cent excess solid lime; and (c) sulfide, sulfhydrate or arsenic sulfide calculated on skin weight for these conditions. All measurements were made at room temperature (23°-25° C). Table 95 and Figure 56 show the pH values obtained with the glass electrode and the calculated values obtained through the use of the Atkin, Goldman, and Thompson equation.

Table 95

Grams S⁼ added to 400 ml lime solution containing 10 gms solid $Ca(OH)_2$	As_2S_3 Glass Electrode	As_2S_3 Calc.	NaHS* Glass Electrode	NaHS* Calc.	Na_2S† Glass Electrode	Na_2S† Calc.
0.00	12.47	12.46	12.47	12 46	12.47	12 46
0.25	12.54	12.43	12.47	...	12 50	....
0.50	12.43	12.41	12.47	...	12.50	...
0.75	12.43	....	12.45	...	12.52	...
1.00	12.43	12.38	12.45	...	12.53	..
1.50	12.38	...	12.45	....	12.57	...
2.00	12.35	12.32	12.44	12.44	12.60	12.58
3.00	12.32	12.26	12.44	12.44	12.68	12.62

* NaHS containing 70% NaHS.
† Na_2S flake containing 60–62% Na_2S.

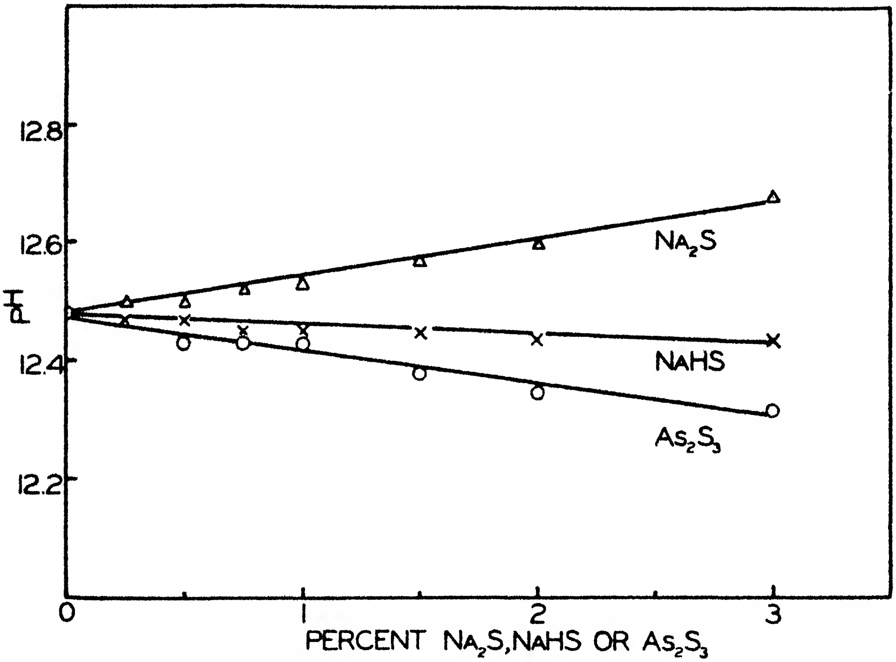


Figure 56. Showing the pH values of unhairing solutions containing $Ca(OH)_2$ with Na_2S, NaHS and As_2S_3.

It is usually assumed that the reactions of Na_2S, NaHS and As_2S_3 with $Ca(OH)_2$ are as follows:

(1) $2Na_2S + Ca(OH)_2 + 2H_2O \rightarrow 4NaOH + Ca(HS)_2$
(2) $2NaHS + Ca(OH)_2 \rightarrow 2NaOH + Ca(HS)_2$
(3) $As_2S_3 + 6Ca(OH)_2 \rightarrow 3CaS + Ca_3(AsO_3)_2 + 6H_2O$

Such equations indicate that Na_2S yields twice as much caustic alkali as does NaHS. The hydrolysis of these two particular compounds should then influence the amount of calcium in solution if $[Ca^{++}] \times [OH^-]^2 = K$. Since Na_2S yields more sodium hydroxide than does the NaHS upon

hydrolysis, the amount of dissolved calcium should be drastically decreased by the addition of Na_2S. Such is found to be the case, as shown by Table 96 and Figure 57. The addition of NaHS up to some 3 per cent, based upon skin weight, does not materially affect the pH value and actually increases the solubility of the lime. However, even small and moderate additions of sodium sulfide markedly affect both the pH value and lime solubility. Many manufacturers of calf and kid leathers maintain that they obtain better leathers by using arsenic sulfide. Tables 95 and 96 show that increasing arsenic sulfide content decreases the pH value and greatly increases the calcium-ion solubility, probably by formation of calcium arsenite. Thus, skins in contact with an unhairing solution containing arsenic sulfide are subjected to the less caustic more mellow action of the dissolved calcium salts.

Table 96

Grams S added to lime solution	Grams CaO per Liter As_2S_3	NaHS	Na_2S
0.00	1.320	1 320	1.320
0.25	1.540	.	.
0.50	1.652	1 424	1.222
1 00	1.982	1.454	1.036
2.00	2 650	1.500	0 774
3.00	3.404	1.536	0 646

The data of Theis and Ricker demonstrate that additions of even 3 per cent sodium sulfhydrate do not materially affect the pH value of the lime liquor but actually increase the solubility of the calcium ion to some extent. Then, in order for the expression $[Ca^{++}] \times [OH^-]^2$ to be a constant, these data would appear to indicate that the usual equation, $2NaHS + Ca(OH)^2 \rightarrow 2NaOH + Ca(HS)_2$, does not obtain in the strict sense, since even small additions of caustic alkali materially decrease the Ca^{++} ion solubility. It would thus seem, since the solubility of the lime increased and the pH value remained essentially constant, that very little sodium hydroxide was found. This may very well be the reason why sodium sulfhydrate acts similarly to arsenic sulfide in the unhairing liquors. Certainly its action is markedly different from that of sodium sulfide.

Many other salts, such as sodium cyanide, sodium sulfite, sodium thiosulfate, sodium sulfate, and sodium carbonate, may be used to alter the pH value and the Ca^{++} ion solubility of an unhairing liquor. Theis and Ricker made a study of the action of these salts when added to a lime liquor. Their reaction may be expressed as follows:

$$2KCN + Ca(OH)_2 \rightarrow 2KOH + Ca(CN)_2$$
$$Na_2S_2O_3 + Ca(OH)_2 \rightarrow CaS_2O_3 + 2NaOH$$
$$Na_2SO_4 + Ca(OH)_2 \rightarrow CaSO_4 + 2NaOH$$
$$Na_2CO_3 + Ca(OH)_2 \rightarrow CaCO_3 + 2NaOH$$
$$Na_2S_2 + Na_2SO_3 \rightarrow Na_2S_2O_3 + Na_2S$$
$$Na_2S_2 + NaCN \rightarrow NaCNS + Na_2S$$

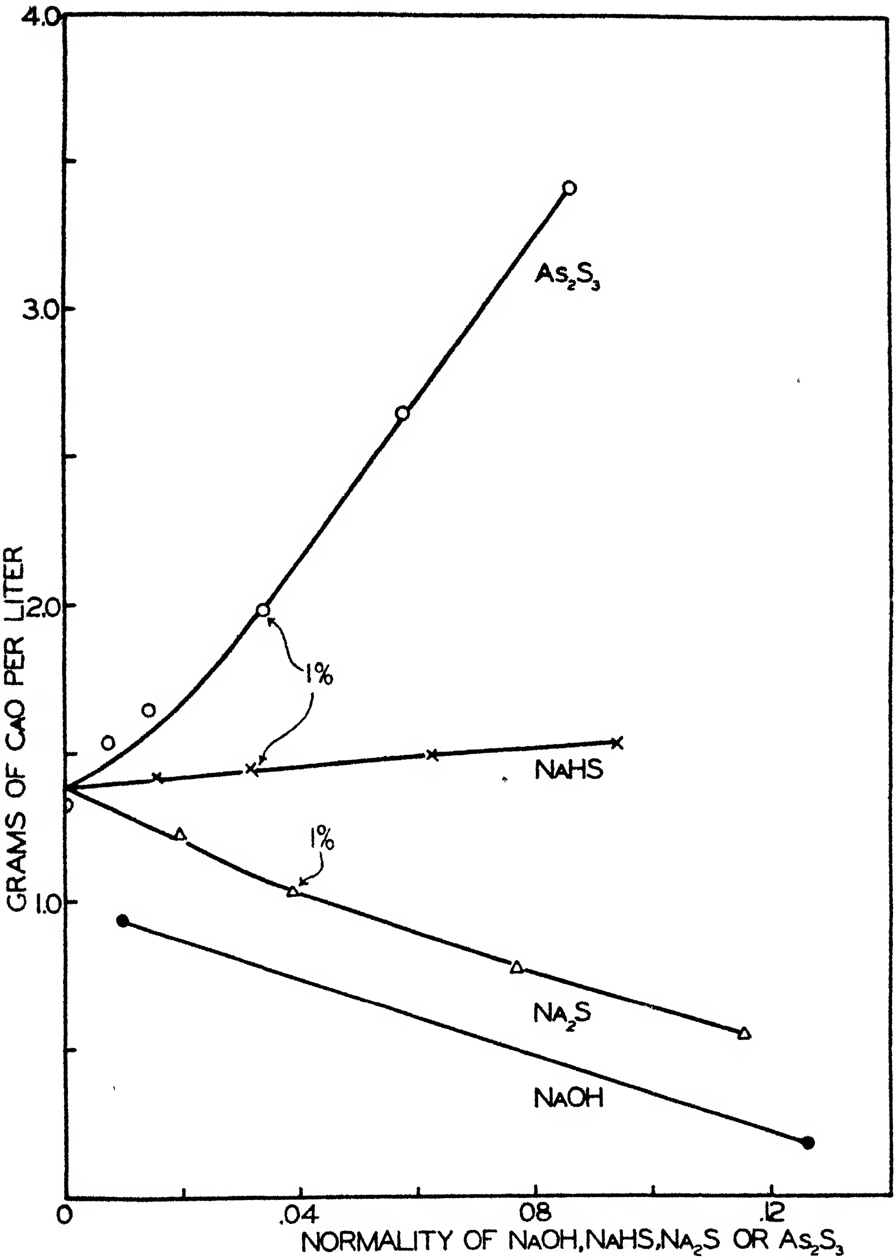


Figure 57. Showing the amount of CaO dissolved in unhairing solutions containing $Ca(OH)_2$ with Na_2S, NaHS and As_2S_3. The arrows indicate the CaO content in solutions containing 1 per cent of sharpener based on skin weight.

The effect of moderate additions of such salts to a lime liquor are shown in Tables 97 to 101, and in Figure 58.

Table 97. Effect of Addition of KCN to 400 ml Lime Liquors Containing 10 gms Solid $Ca(OH)_2$.

KCN* added (gram)	pH	Grams soluble CaO/liter
0.00	. . .	. . .
0.25	12.50	1.424
0 50	12.51	1.424
0 75	12 53	1.410
1.00	12.53	1.406

* Amount of cyanide added corresponds to actual percentage based on green salted skin weight in a liquid-skin ratio of 4:1.

Table 98. Effect of Additions of Na_2SO_3 to 400 ml Lime Liquor Containing 10 grams Solid $Ca(OH)_2$.

Na_2SO_3 added (grams)	pH	Dissolved calcium as grams CaO/liter
0.00	12 50	1.400
.25	12.57	1.251
.50	12.60	1.096
.75	12.62	0.948
1.00	12.60	0.812
2.00	12.76	0.526
3.00	12.86	0.366
4 00	12.95	0.261
5.00	13 01	0.213
6 00	13.07	0.177

Table 99. Effect of Addition of $Na_2S_2O_3$ to 400 ml Lime Solution Containing 10 grams Solid $Ca(OH)_2$.

$Na_2S_2O_3$ added (gram)	pH	CaO (grams per liter)
0.00	12.53	1.400
0.25	12.53	1 443
0.50	12.53	1 487
0.75	12 53	1.513
1.00	12 53	1.535

Table 100. Effect of Moderate Additions of Na_2SO_4 to 400 ml Lime Liquor Containing 10 grams Solid $Ca(OH)_2$.

Na_2SO_4 added (gram)	pH	CaO (grams per liter)
0.00	12.53	1.400
0.25	12.53	1.436
0.50	12.53	1.487
0.75	12.53	1 520
1.00	12.53	1.576

Table 101. Effect of Addition of Na_2CO_3 to 400 ml Lime Liquor Containing 10 grams Solid $Ca(OH)_2$.

Na_2CO_3 added (gram)	pH	CaO (grams per liter)
0.00	12.53	1.400
0.25	12.56	1 208
0.50	12.58	1.010
0.75	12.61	0.854
1.00	12.63	. .

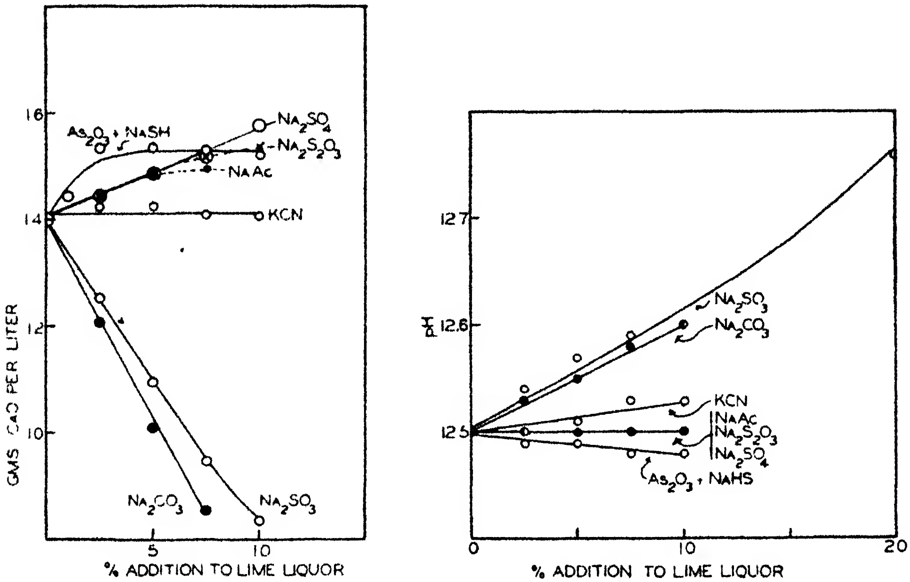


Figure 58. The effect of addition of various salts to a lime suspension upon the pH value and soluble calcium.

It has been pointed out previously that the cyanide salts are very efficient as "sharpeners" in the unhairing action and that they are, in general, more active than the sulfides. As shown in Table 97, moderate additions of the alkali cyanides have but little effect upon either the pH or the Ca^{++} ion solubility of the lime liquor. It seems, therefore, that the reaction $2NaCN + Ca(OH)_2 \rightarrow Ca(CN)_2 + 2NaOH$ does not occur to any great extent. When cyanides are used in conjunction with lime, it appears that the reaction with the keratins is a breakdown of the disulfide linkage, with consequent formation of sulfocyanate. Salts such as the thiosulfate, sulfate and carbonate are not, as a rule, used in the unhairing bath, and have been discussed to some extent elsewhere.

In 1942, Theis and Blum[42] investigated the effect of adding calcium sulfhydrate to the lime liquors. This investigation was carried further than their previous work and note was taken of unhairing time. Their data are shown in Table 102 and Figure 59.

Table 102. Showing pH and CaO Solubility in Systems $Ca(OH)_2$—$Ca(HS)_2$

Grams $Ca(HS)_2$ in 400 ml soln	No $Ca(OH)_2$ added		10 gms excess $Ca(OH)_2$ added		D-B	E Unhairing Time	
	A pH	B Gms CaO/ 100 ml	C pH	D Gms CaO/ 100 ml		Long hair	Short hair
0.00	....	.....	12.61	0.1374	0.1374	7 days	7 days
0.25	9.28	0.0389	12.57	0.1707	0.1318	3 days	3 days
0.50	9.43	0.0788	12.54	0 2051	0.1263	68 hrs	60 hrs
0.75	9.66	0.1155	12.51	0.2395	0.1240	24 hrs*	36 hrs
1.00	9.92	0.1554	12.49	0.2734	0.1180	24 hrs*	24 hrs
2.00	10.28	0.3108	12.40	0.4235	0.1127	24 hrs*	24 hrs †
3.00	10.55	0.4669	12.33	0.5783	0.1114	....	....
4.00	10.69	0.6236	12.30	0.7317	0.1081	...	...
5.00	10.82	0.7804	12.24	0.8878	0.1074	24 hrs*	24 hrs †

* Long hair mushed and rotten.
† Short hair not being attacked readily.

These data show that additions of calcium sulfhydrate cause a decreased pH value and an increased Ca^{++} ion content. Column A indicates that the pH value of aqueous solutions of calcium hydrosulfide increases with increasing concentration; this in all probability being due to the reaction:

$$HS^- + HOH \rightarrow H_2S + OH^-$$

However, when calcium hydrosulfide is added to a lime solution (pH = 12.61), the alkalinity of the sulfhydrate is not sufficient to affect that of the lime solution. However, a second reaction takes place, namely, the conversion of a portion of the HS^- ion to the insoluble $S^=$ ion, bringing about a lessened $Ca^=$ ion content (column D-B) and a lessened pH value, as shown in column C.

In the discussion dealing with arsenic trisulfide, it was postulated that the reaction of arsenic trisulfide with lime might be:

$$2As_2S_3 + 9Ca(OH)_2 \rightarrow 3Ca(HS)_2 + 2Ca_3(AsO_3)_2 + 6H_2O$$

Therefore it would appear that if calcium sulfhydrate and calcium arsenite were mixed in the proportion of 3 mols sulfhydrate and 2 mols arsenite, the same conditions should obtain as when arsenic sulfide was used. Theis and Blum, using As_2O_3, $Ca(OH)_2$ and $Ca(HS)_2$ in the proper ratio, as indicated by the equation, limed properly soaked skin in such a liquor. They noted the pH value of the liquor and also the time necessary for unhairing. Their results are shown in Table 103. These data show that the SH^- ion alone is not sufficient for unhairing and that the OH^- ion is also necessary. This is particularly to be noted as the percentage of As_2S_3 increases, thus lowering the pH value. When the latter is less than 12, the unhairing action is retarded, as can readily be seen from Table 103.

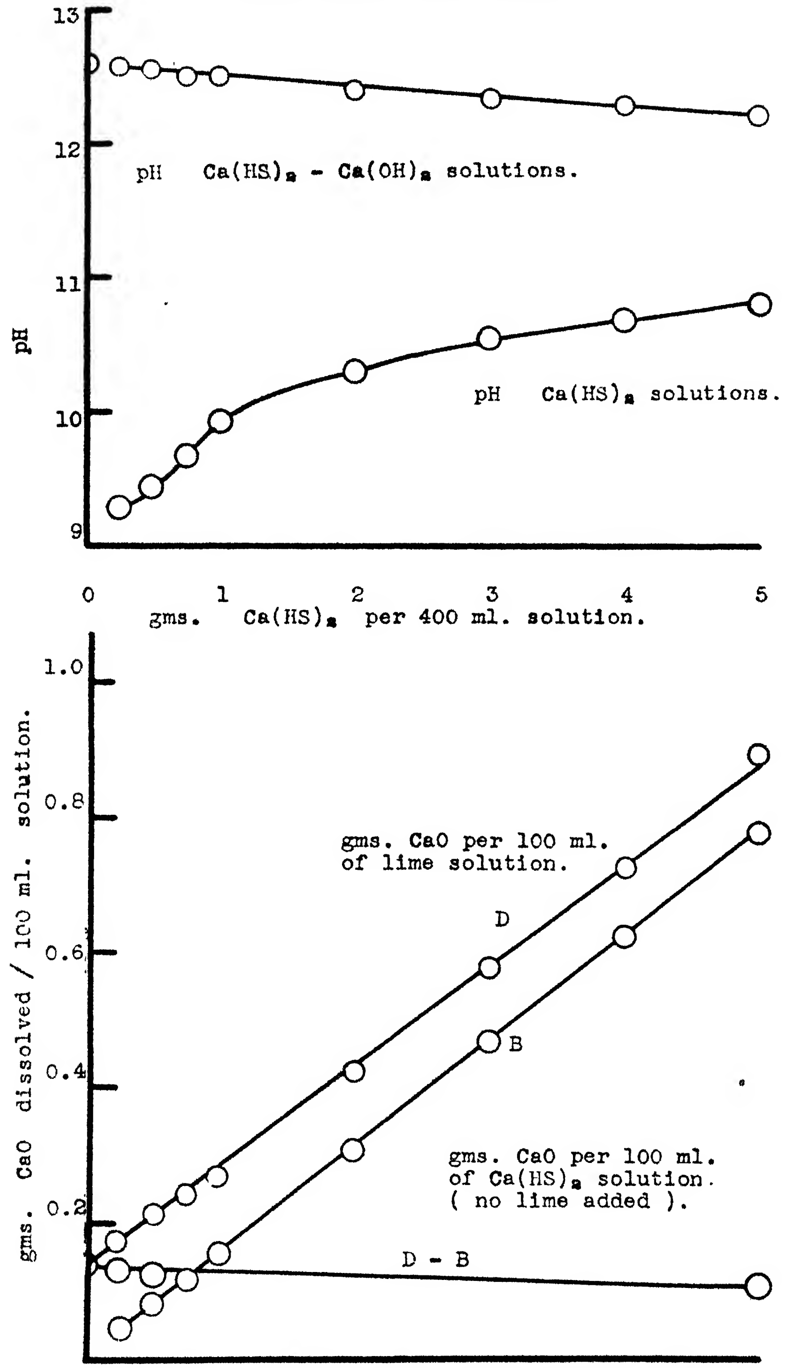


Figure 59. The effect of $Ca(HS)_2$ upon the pH value and upon the dissolved calcium of unhairing solutions.

Table 103

% As_2S_3 added	pH Before Unhairing	pH After Unhairing	Unhairing Time Long hair (hours)	Unhairing Time Short hair (hours)
0.5	12.38	12.32	12	72
1.0	12.30	12.27	12*	72
1.5	12.20	12.19	12*	72
2.0	11.90	11.87	120*	240

* Long hair mushed and rotten.

% As_2O_3 equivalent to As_2S_3†	pH Before Unhairing	pH After Unhairing	Unhairing Time Long hair (hours)	Unhairing Time Short hair (hours)
0.5	12.38	12.23	12*	72
1.0	12.31	12 20	12*	72
1.5	12 18	12.01	24*	240

* Long hair mushed and rotten.
† Solutions made by adding As_2O_3, $Ca(HS)_2$ and $Ca(OH)_2$ to give the equivalent of As_2S_3 percentages noted.

Theis and Blum performed further experiments using calcium sulfhydrate, which was made by passing H_2S gas into a water suspension for periods ranging from 1 to 60 minutes. The calcium hydroxide-sulfhydrate solutions were then used for unhairing experiments. The data are given in Table 104.

Table 104

H_2S passed (min)	Grams† $Ca(HS)_2$ formed per 100 ml soln	pH Before Unhairing	pH After Unhairing	Unhairing Time Long hair	Unhairing Time Short hair	Grams CaO/100 ml soln
0	0.000	12.56	12.40	7 days	7 days	0.1350
1	0.021	12.51	12.38	5.5 days	5.5 days	0.1470
5	0.077	12.45	12 40	1.5 days	1.5 days	0.1900
10	0.191	12.41	12.37	12 hrs*	36 hrs	0.2360
15	0.247	12.38	12.32	12 hrs*	36 hrs	0.2870
30	0.449	12.31	12.22	12 hrs*	60 hrs	0.4240
45	0.741	12.20	12.10	12 hrs*	96 hrs	0.6060
60	1.132	12.15	12 00	12 hrs*	100 hrs	0.8300

* Long hair mushed and rotten.
† For percentage $Ca(HS)_2$ based on hide weight multiply by 4.

This table shows that as the pH value decreases, due to increase of $Ca(HS)_2$, actual short hair unhairing is retarded. Table 104 shows that this condition exists when a concentration of $Ca(HS)_2$ greater than 1.5 per cent is used. As the concentration is increased beyond 1.5 per cent, the fine hair, scud, and other keratinous substances are difficult to remove. Part of the unhairing difficulty is undoubtedly due to the decreased pH of the unhairing solution. That such conditions obtain are shown more in detail in the following investigations of Theis and Blum. Pieces of well soaked steer hide were placed in the following solutions for 24 hours at 20° C.

	Solution	pH
(1)	NaOH	10.0
(2)	NaOH	10.5
(3)	NaOH	11.0
(4)	NaOH	11.5
(5)	NaOH	12.0
(6)	Saturated $Ca(OH)_2$ containing solid lime	12.5
(7)	NaOH	12.5
(8)	Same as (6) but adjusted with NaOH	13.0
(9)	NaOH	13.0

After 24-hour treatment in these solutions, the skin was removed and placed in unhairing solutions containing 6 per cent hydrated lime, 1.0 per cent $Ca(HS)_2$ and a volume ratio, liquid to skin, of 4 to 1. The strips of skin were tested for unhairing after each 12-hour period. The unhairing solutions were maintained at 20° C. The data are given in Table 105.

Table 105

pH of alkaline soak	Out of alkaline soak	Unhairing Condition: After 24 hrs in $Ca(OH)_2$—$Ca(HS)_2$ soln	Unhairing Condition: After 48 hrs in $Ca(OH)_2$—$Ca(HS)_2$ soln
(1) 10.0	− − −	− − −*	+ + +
(2) 10.5	− − −	− − −*	+
(3) 11.0	− − −	− − −*	− − −*
(4) 11.5	− − −	+*	+*
(5) 12.0	− − −	+*	+ + +*
(6) 12.5	+	+ +	+ +
(7) 12.5	+	+ +	+ + +
(8) 13.0	+ +	+ + +	+ + +
(9) 13.0	+ +	+ +	+ + +

* All hair mushed

Table 106 shows additional data using the same preliminary treatment, but for reliming 0.5 per cent $Ca(HS)_2$ was used in place of the 1.0 per cent employed for the data given in Table 105.

Table 106 shows that the pH value of the final unhairing solution has much to do with the unhairing when excess $Ca(HS)_2$ is used. It is necessary to have a sufficient amount of alkali present to counteract the effect of the

Table 106

pH of alkaline soak	Out of alkaline soak	Unhairing Condition: After 24 hrs in $Ca(OH)_2$—$Ca(HS)_2$ soln	Unhairing Condition: After 48 hrs in $Ca(OH)_2$—$Ca(HS)_2$ soln	Unhairing Condition: After 72 hrs in $Ca(OH)_2$—$Ca(HS)_2$ soln
(1) 10.0	− − −	− +	+ + +	
(2) 10.5	− − −	+	+ + +	
(3) 11.0	− − −	+	+ + +	
(4) 11.5	− − −	+	+ + +	
(5) 12.0	+	+	+ + +	
(6) 12.5	+	− +	+ +	+ + +
(7) 12.5	+	− +	+ +	+ + +
(8) 13.0	+ +	+ +	+ +	+ + +
(9) 13.0	+ +	+ +	+ +	+ + +

reduction in pH value caused by the excess $Ca(HS)_2$. Employing such high concentrations of $Ca(HS)_2$ as used for the experiments in Table 105 counteracts immunization reactions.

Table 106, however, shows nicely the immunization caused by high alkalinity. Whereas skin pretreated at pH values lower than 12.5 unhaired in 48 hours, using 0.5 per cent $Ca(HS)_2$, skin pretreated at higher pH values did not unhair until 72 hours had elapsed, although the hair had been loosened two-thirds of the way in the alkaline pretreatment bath. In other words, the high alkalinity had caused such a change in the keratin matter of the hair that it was resistant to the SH^- ion.

Theis and Blum investigated the effect of various unhairing liquors upon the shrinkage temperature of the limed skin. Goat skin was used for this work. The lime liquors were made up using 3 grams hydrated lime plus the additional "sharpeners" noted and 200 ml water. The strips of skin used were allowed to remain in contact with the specified lime liquor from 1 to 10 days. After such treatment the skin was unhaired, washed, and the shrinkage temperature determined. The results so obtained are given in Table 107. These data show that even dilute sodium sulfide solutions cause

Table 107. Effect of Various Unhairing Agents Upon the Shrinkage Temperature of Skin.

Salt added to saturated lime	Conc g/200 ml†	Original pH of solution	Shrinkage Temperature				
			After 24 hrs	After 48 hrs	After 72 hrs	After 120 hrs	After 240 hrs
Blank H_2O None	..		65.4	64.2	64.9	65.4	67.0
Sat. $Ca(OH)_2$	0.0	12.58	58.0	57.1	57.0	55.1	55.4
NaCN	0.5	12.40	59.5	58.3	53.1	51.7	51.7
NaHS	0.5	12.49	56.7	56.0	58.4	55.2	51.5
$Ca(HS)_2$	0.5	12.50	58.4	57.1	57.7	55.4	52.0
Na_2SO_3	2 0	12.91	53.5	52.0	50.7	46.3	48.3
As_2S_3	0.5	12.41	59.2	57.5	58.8	58.2	51.7
Na_2S	0.5	12.60	54.0	53.6	53.3	52.2	50.8
Na_2S	1.0	12.65	52 0	52.5	49.9	50.0	48.3
Na_2S	2.0	12.80	51.6	47.8	49.5	50.1	45.8
Na_2S	3.0	12.85	50 5	49.5	44.3	44.8	44.4
Na_2S	5.0	13 00	46.6	45.5	43.3	44.5	...*

* Structure broken down.
† To obtain per cent unhairing agent (based on soaked weight) multiply by 2.

drastic changes in the shrinkage temperature of the limed skin, especially during the early period of liming. Sodium or calcium sulfhydrate appears to cause little change in this factor when compared to that caused by a straight limin process. The data obtained indicate that the use of arsenic sulfideg as an unhairing adjunct causes little or no breakdown in the skin structure.

Swelling and Plumping

Marriott,[21] in 1932 and 1933, studied the swelling of collagen fiber bundles in acidic and alkaline solutions. Single fiber bundles were held at both ends and then treated with solutions of hydrochloric acid in the pH range 0.3 to 5.0. The alterations were measured by means of photomicrographs. Marriott found that swelling commenced at about pH 3.5 and postulated that the first increase in volume was permanent, and was due to the "opening up" of the structure. He believed that later volume increases were due to

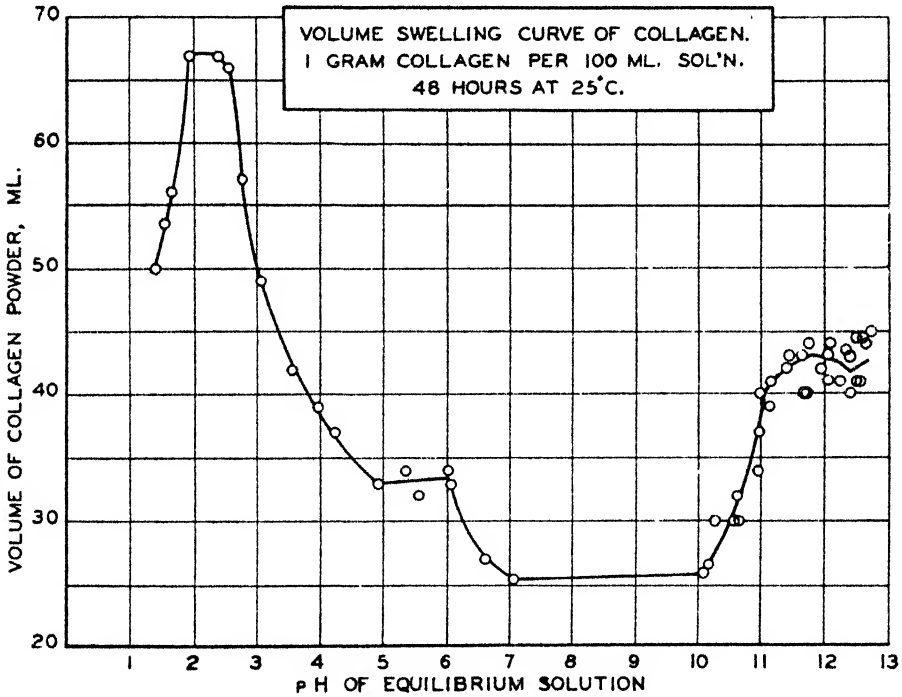


Figure 59A

plumping. He pointed out that swelling, or "opening up," is indicated by an increase in the width of the film without any alteration in its length, whereas plumping or "osmotic take up" is indicated not only by an increase in width but by an accompanying decrease in film length. Lloyd, Marriott, and Pleass[18] showed that alkaline swelling does not begin at pH values immediately greater than the isoelectric point, but first becomes definite at pH 9.5. These investigators found a suggestion of a swelling maximum at pH 10.5 followed by a definite increase in volume at higher pH values. Swelling in sodium hydroxide solutions was greater than in calcium hydroxide solutions of the same pH value.

In 1936, Highberger[13] investigated the volume and weight gain of purified collagen. For this work he used unbuffered solutions in the pH range 1.5 to 12.5. His data are shown in Figures 59A and 59B. The two curves are quite similar in trend and display some resemblance to the acid-base titration curve for collagen. The accepted isoelectric point at pH 4.9 is not well defined in either of the curves, but both show a flattened portion in the pH range 4.9 to 6.0. Beginning at pH 6.5, the curves descend sharply to attain a broad minimum value in the pH range 7.0 to 10.1. Alkaline swelling begins at about pH 10.5, increasing to about pH 12.0, and showing an apparent minimum value at approximately pH 12.3. Porter[38] earlier showed

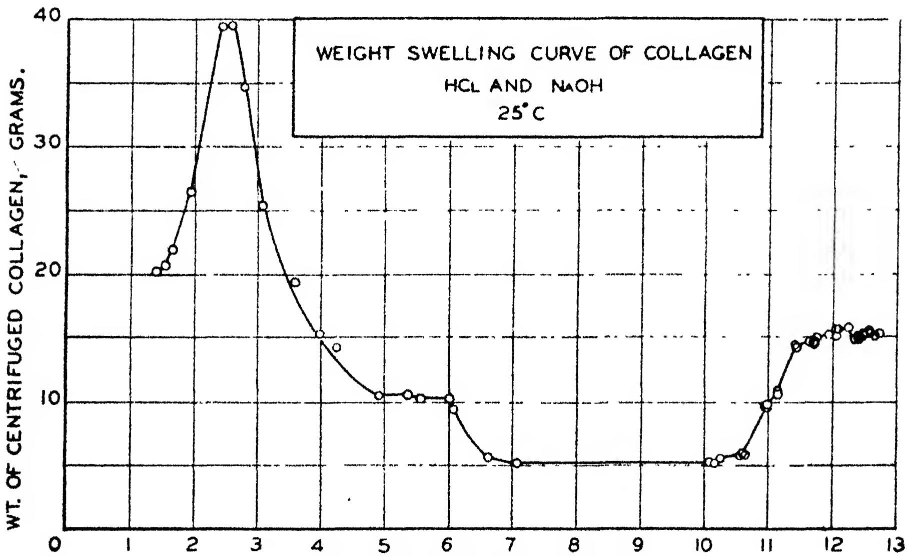


Figure 59B

a similar slight minimum in the alkaline swelling curve of hide powder. Highberger points out that it is perhaps premature to speculate upon the significance of three minimum values in the complete swelling curve of collagen. It is rather inconceivable that a single molecular species of ampholyte can have more than one isoelectric point. The existence of the second and now the third isoelectric point can only point to the possibility of a second and third molecular species. Highberger goes on to state that the material must have consisted originally of a mixture of such species, or the several species must have been produced from the original chemically homogeneous substance through the action of the various reagents used for obtaining the several isoelectric points. The findings of Wilson and Gallum[51]

and of Wilson and Kern[51] in this regard have been adequately discussed in Volume I of the second edition of this monograph. As pointed out by Highberger, the existence of the third isoelectric point at approximately pH 12.3 might be accounted for by assuming that the tautomeric change is carried further in strongly alkaline solutions and thus results in the production of a third molecular species.

It is still a matter of speculation as to whether or not the three minimum points of swelling, as noted, actually represent true isoelectric points. In view of the findings of Beek and Sookne, Highberger, and Theis and Jacoby relative to the true isoelectric point of collagen, namely in the proximity of pH 7.6, it will require demonstration by methods of electrophoresis and electroendosmosis to make certain that the three minimum values represent isoelectric points.

Table 108. The Effect of Change in Concentration of NaOH upon Dimensions of Fibres.

pH (Calc.)	Concentration	Fresh Fibers						Dry Fibers					
		Y		M		O		Y		M		O	
		Length	Width	Length	Width	Length	Width	Length	Width	Length	Width	Length	Width
14.1	N/1	Disintegrated		Disintegrated		Disintegrated		Disintegrated		Disintegrated		Disintegrated	
13 6	N/3	60.5	730	80 4	845	75.7	673	69.6	700	68.5	862	94.4	815
13 1	N/10	71.0	533	71 3	557	72.1	858	80	500	80.7	510	77.2	500
12 6	N/30	73 8	463	75	500	80	430	87.5	350	89	295	81.3	455
12.1	N/100	77.6	426	77 3	396	74.3	437	88.6	520	86.2	360	82.1	335
11.6	N/300	78.4	506	78.8	491	79 2	550	91	270	89.2	188	83	326
11 4	N/500	92.0	244	88	421	88.9	270	91.5	198	98.2	222	87	182
11.1	N/1000	89.8	337	76	513	83.4	416	101.2	148	101.9	138	98.5	120
10.8	N/2000	98.4	188	99 3	113	98.5	140	102.3	115	101.8	118	100	108
10 4	N/5000	95.5	128	100	114	98 5	110	101	138	100	112	100	104
10.1	N/10000	100	104	100	100	100	100	100	150	100	100	100	100
9.8	N/20000	100	100	100	100	100	100	100	100	100	100	100	100

In 1937, Lloyd and Stockall[20] studied the swelling of structured proteins in alkaline solutions. For this investigation, they used tendons from the tails of rats. The alkaline solutions were sodium hydroxide, calcium hydroxide, and sodium sulfide; 5-mm lengths of tendons were used for each 50 ml of alkali. Measurements of length and width were made on photomicrographs of the swelled tendon. The results for alkali swelling are shown in Tables 108, 109, and 110.

Commenting upon these data, Lloyd and Stockall state: (1) that the maximum swelling obtained in sodium hydroxide solutions is only about half that obtained in hydrochloric acid solutions; (2) that swelling does not begin immediately on the alkaline side of the isoelectric point, but at approximately pH 10.5; (3) that swelling in calcium hydroxide solutions is less than in either sodium hydroxide or sulfide, but continued for more than a week

Table 109. The Effect of Change in Concentration of Na_2S upon the Dimensions of Fibers.

pH (Calc.)	Concentration	Fresh Fibers						Dry Fibers					
		Y		M		O		Y		M		O	
		Length	Width	Length	Width	Length	Width	Length	Width	Length	Width	Length	Width
13.8	M/1	Disintegrated		93.25	1260	Disintegrated		62.25	1053	62	900	77.7	1300
13.3	M/3	64.7	1482	64.5	707	60.7	1180	79.7	550	67.25	1086	70.25	604
12.8	M/10	73.5	368	70.2	450	71.5	572	85.75	406	76	613	78.4	429
12.3	M/30	76.5	462	81.2	438	76 7	563	88.1	294	85.25	446	82.25	574
12.1	M/50	82	335	81.2	600	82.2	520	87.25	436	80	472	79.4	515
11.8	M/100	74.6	582	81.6	530	78	456	87.25	405	83.3	530	81.75	404
11.3	M/300	99.25	164	91.5	229	97 7	205	97.25	530	92 75	367	85.75	200
11.1	M/500	100	199	96.25	139	99	129	98.9	173	98.4	131	99	180
10.8	M/1000	99.25	119	99.25	155	98.5	134	100		100	124	100	131
10.3	M/3000	98.5	102.7	100	104.5	100	91.5	100	153	100	103	100	86.5
10.1	M/5000	100	100	100	113	99.5	100	100	131	100	100	100	109
9.8	M/10000	100	100	100	100	99 2	91	100	100	100	100	100	75.2

Table 110. Effect of Change in Concentration of $Ca(OH)_2$ upon the Dimensions of Fibers.

pH (Calc.)	Concentration	Fresh Fibers						Dry Fibers					
		Y.		M.		O.		Y.		M.		O.	
		Length	Width	Length	Width	Length	Width	Length	Width	Length	Width	Length	Width
12.8	Sat.	102	280	106	274	104	148	100	165	103	153	102	152
12.7	N/25	102	120	102	150	101	102	101	141	100	165	101	137
12.4	N/50	98	150	100	150	101	155	100	122	99	141	101	145
12.1	N/100	109	140	96	141	100	129	103	131	100	176	100	136
11.8	N/200	100	150	99	131	100	157	103	133	101	100	99	100
11.4	N/500	100	154	97	123	100	122	100	121	100	109	99	100
11.1	N/1000	100	126	98	137	100	108	100	106	101	100	98	100
10.8	N/2000	99	144	100	134	100	108	101	141	101	102	100	127
10.4	N/5000	95	180	100	127	100	166	...	...	.	...	..	..
10.1	N/10000	100	153	98	163	100	116	100	100	100	105	100	100

without attaining equilibrium; and (4) that alkaline pretreatment with sodium hydroxide or sulfide weakens the reticular tissue.

In 1938 Fritsch[9] studied the influence of the liming system upon the physical properties of calf leather. At this point only the data relating to the swelling factor will be discussed and the reader is referred to the original literature for the more practical aspects of this work. Fritsch first investi-

Table 111. Sodium Hydroxide.

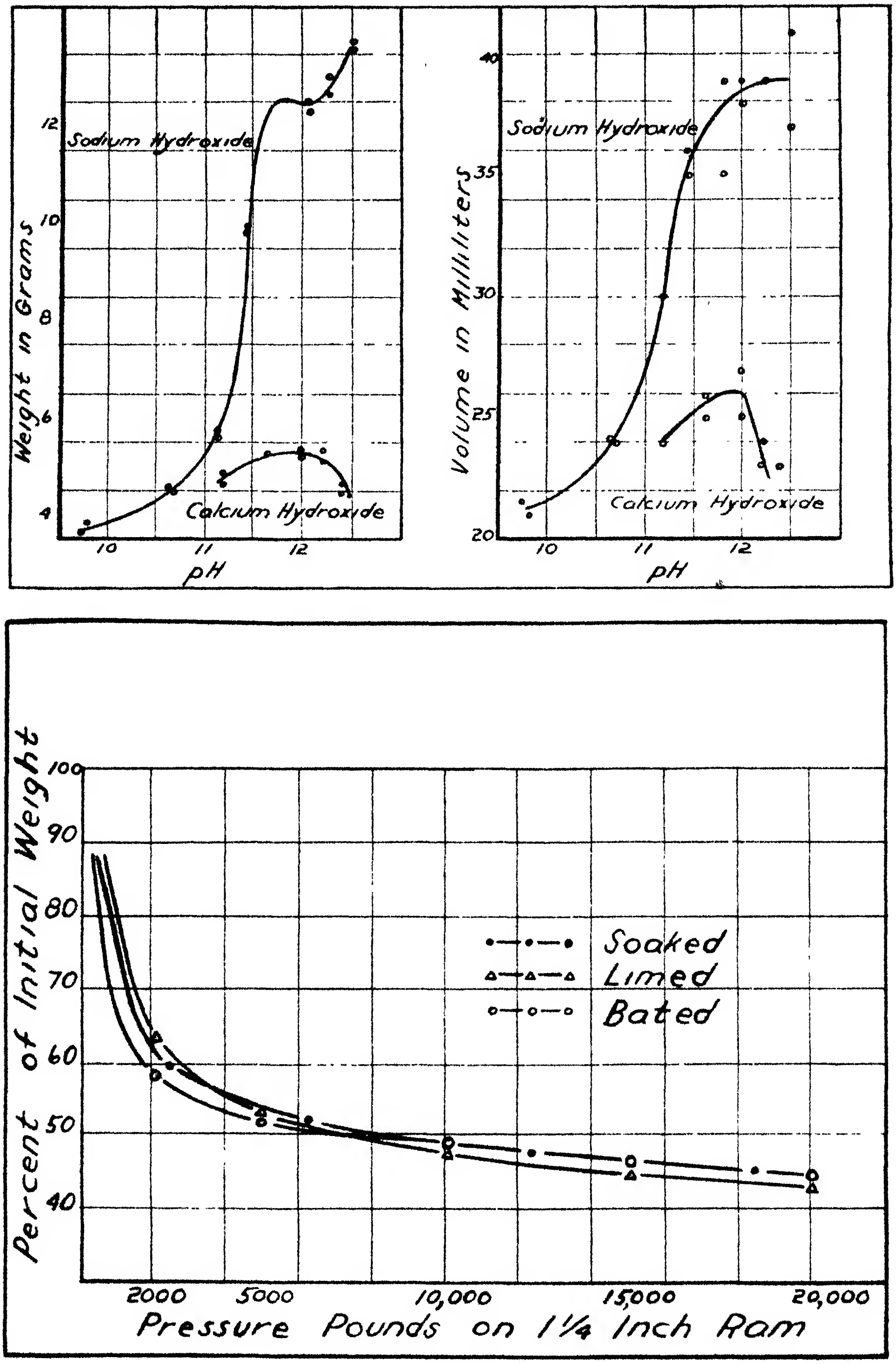
Initial pH	pH After 48 hours	Volume (ml)	Weight (grams)	pH After 48 hours	Volume (ml)	Weight (grams)
12.64	12.50	41	13.68	12.50	37	13.78
12.35	12.24	39	12.65	12.23	39	13.04
12.18	12.03	39	12.55	12.03	38	12.30
11.90	11.80	39	12.49	11.80	35	12.60
11.52	11.42	36	9.90	11.44	35	9.88
11.40	11.15	30	5.56	11.15	30	5.63
11.23	10.64	24	4.54	10.69	24	4.46
10.90	9.75	21.5	3.64	9.80	21	3.68

Table 112. Calcium Hydroxide.

pH After 48 Hours	Volume (ml)	Weight (grams)	pH After 48 Hours	Volume (ml)	Weight (grams)
11.20	24	4.58	11.20	24	4.86
11.63	25	5.29	.	26	..
12.01	27	5.26	12.01	25	5.29
12.21	24	5.32	12.21	23	5.10
12.40	23	4.49	12.41	17	4.62

gated the volume and weight gain in a manner similar to that used by Highberger. In this work, he studied both the sodium and calcium hydroxide systems. His data are shown in Tables 111 and 112 and in Figures 60 and 61.

Fritsch found that swelling in calcium hydroxide was less than in sodium hydroxide and thus corresponded to the valency rule postulated by Loeb, Wilson, and others. In subsequent study, he investigated the swelling in



Weight in Grams
12
10
8
6
4
Sodium Hydroxide
Calcium Hydroxide
10
11
12
pH
Volume in Milliliters
40
35
30
25
20
Sodium Hydroxide
Calcium Hydroxide
10
11
12
pH
Percent of Initial Weight
100
90
80
70
60
50
40
Soaked
Limed
Bated
2000
5000
10,000
15,000
20,000
Pressure Pounds on 1¼ Inch Ram

Figure 60

mixtures of sodium and calcium hydroxide at a constant pH of 12.4 and found that although the swelling of collagen did not change materially with the pH value (11.2 to 12.4), it did change considerably with the ratio of calcium

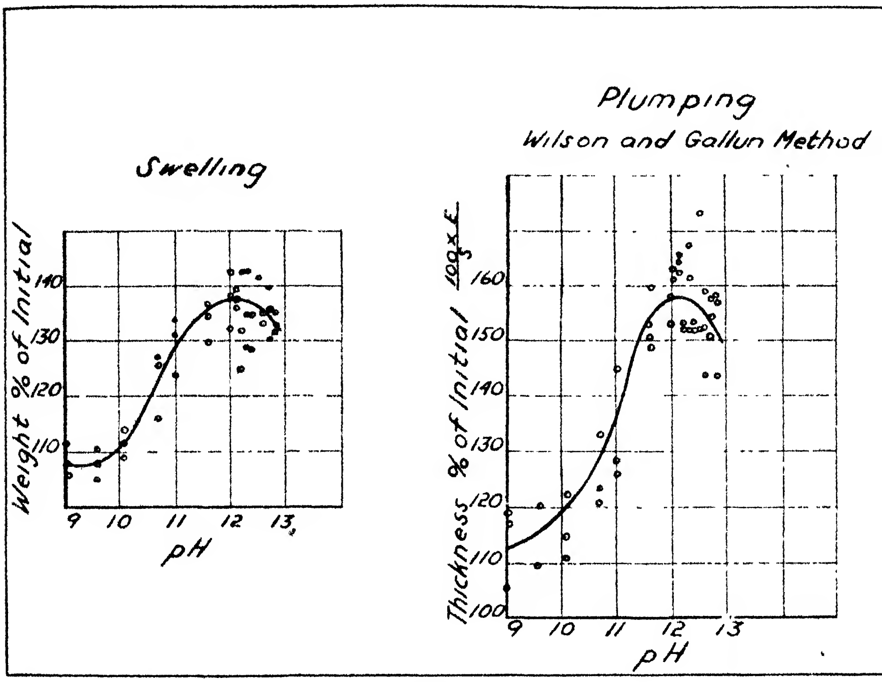


Figure 61

hydroxide to sodium hydroxide. These additional data are shown in Table 113.

Table 113

Lime (ml)	Sodium Hydroxide (ml)	pH After 60 Hours	Volume (ml)		Weight (grams)	
100	...	12.40	25	25	5.51	5.82
80	20	. .	26	28	5.88	6.34
60	40	12.37	28	27	6.66	6.85
40	60	. . . .	31	28	8.68	8.40
20	80	12.33	35	34	13.00	12.74
...	100	12.40	40	40	15.39	15.91

Fritsch investigated further the plumping and swelling of steer hide. For these data, he estimated the plumping by measuring the decrease in weight under increased pressure. The basis of this method is the assumption that the difference between plumping and swelling involves a variation in the manner in which water is held in the protein. Fritsch, therefore, reasoned

that the rate at which water is expelled from the treated protein under pressure should vary in relation to the plumpness. His data are shown in Tables 114 and 115, and in Figure 60.

Table 114. Soaked Hides.

Pressure (lbs per sq inch)	Pressure on 1.25 ram	Weight (grams)	Per cent of initial weight
....	....	5.40	100.0
2,000	2,455	3.23	59.7
5,000	6,130	2.82	52.2
10,000	12,280	2 58	47.8
15,000	18,410	2.44	45.2
....	dry	...	30.7

Table 115

Pressure (lbs on 1 25 ram)	Limed Hides Weight (grams)	Limed Hides Per cent of initial weight	Bated Hides Weight (grams)	Bated Hides Per cent of initial weight
.....	5.39	100.0	4.60	100.0
2,000	3.44	63.8	2.68	58.3
5,000	2.88	53.4	2.42	52.6
10,000	2.60	48.2	2.23	48.5
15,000	2.40	44.5	2.12	46.1
20,000	2.28	42.3	2.04	44.4
dry	1.46	27.1	1.38	30.0

The three curves shown in Figure 60 indicate no real difference between the soaked, limed, and bated states. Fritsch suggests that his results appear to indicate that under high pressure the difference in plumping disappears and therefore the forces causing the plumping are of a low order of magnitude.

In a series of papers, Lloyd[19] *et al.* discuss the binding of water by gelatin and collagen and the relation of such binding to plumping and swelling. They point out that collagen fibers can absorb water under two independent sets of forces, the one due to a formation of a Donnan equilibrium which is controlled by the pH value of the system and the other due to a Hofmeister effect of the acids, salts and bases, each of which produces its own characteristic effect. Lloyd *et al.* presume that the Donnan effect is osmotic in nature and leads to the free water in the system, whereas the Hofmeister effect tends to give increased water binding. Lloyd and Moran show that pressures under 8,000 pounds per square inch remove only the free water of the system and a force greater than 38,000 pounds per square inch is necessary to affect the bound water. Lloyd and Marriott postulate that the action of alkalies upon collagen can be divided into a Donnan and a lyotropic effect. The Donnan effect, or plumping, appears to be definite in the case of sodium or potassium hydroxide but is not always evident in the case of calcium hydroxide. The Donnan effect is latent over the pH range 5.0 to 11.2. The Hofmeister effect becomes evident at about pH 9.0 and both effects are apparent at the regular liming pH value of the unhairing bath.

Liming of skins and hides must then be considered a process necessary not only for the loosening and removal of hair but also for conditioning the skin or hide for the subsequent processes of bating, pickling and tanning.

During the liming process, the skin binds and imbibes water and becomes plumped and swollen, and reacts with the alkalies present to form "proteinates." In addition, the alkali reacts with the keratinous proteins of the hair and epidermis, causing definite degradation of these substances. At the stage where the hair slips satisfactorily, the skins are removed from the unhairing liquor, the hair is removed from the skins by mechanical means, and the skins are then ready for the next operations, known as deliming and bating, and which will be discussed in the next chapter.

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Chapter 10

Bating

In its strict sense, the modern term "bating" refers to the action of proteolytic enzymes upon a skin or hide following the unhairing process, described in the preceding chapter. The bating process is thus quite distinct in character from the mere "deliming" of the skin by means of acids or salts, although, as we shall see, deliming is a necessary part of bating.

When a skin leaves the lime unhairing solution and has been mechanically treated to remove hair, epidermis, flesh, fat and surface muscles, it is in a highly swollen state and has a pH value of about 12.5. Many centuries ago tanners learned that if such a swollen skin were treated with an aqueous infusion of fowl or dog dung prior to tanning, certain desirable characteristics were found in the finished leather. These characteristics were lacking if this weird treatment was omitted. The leather properties imparted by bating cannot even today be expressed by numerical values, they cannot be adequately described in words, and it is difficult to recognize them by microscopic means. But the experienced tanner or shoe manufacturer knows them by the smoothness and silkiness of the grain and a certain desirable "feel" of leather which has been bated; these qualities are lacking in unbated leather. Until some thirty years ago, bating was performed entirely with manure; when hen or pigeon manure was employed the process was termed "bating," and when dog manure was used it was called "puering." Fowl manure was usually employed for heavy hides since it rapidly penetrated the hide, possibly because of its content of urinary products; dog manure was used for light skins. Regardless of the type of manure employed, the process was filthy, disgusting and unhealthy. These conditions led to some of the most remarkable work in the annals of leather science—the investigations and conclusions of J. T. Wood, and their application by Otto Röhm.

In 1898, Wood[24] published a paper on the constitution and action of manure bates; and this was followed by a long series of brilliant and painstaking investigations, which may be summarized as follows. The mineral matters present in manure bates were found to be chiefly sulfates, chlorides and phosphates of sodium, potassium, ammonium, calcium and some silica. The more important organic materials were bacteria, enzymes, cellulose matters and fats. He found both peptic and tryptic enzymes, a rennin, an amolytic enzyme and a lipase. (Those readers who are interested in the

early studies of bating are referred to the book written on this subject by Wood,[25] in 1912, in which are included detailed references to the work of his contemporaries.) Wood established that the essential principle of the manure bate was the proteolytic enzymes it contained. This finding led to the manufacture of "synthetic" bates which rapidly displaced the use of manure; so far as we know, there are no manure bates employed in the United States today. There are many types of synthetic bates, but all essentially consist of enzymes, an inert material (such as wood flour or sawdust, which serves as an enzyme adsorbent) and neutral deliming salts. The source of the proteolytic enzymes used in bate manufacture may be bacterial or fungal, or they may be extracted from the pancreas of animals, such as the sheep or hog. The latter source was the one employed by Röhm[15] in the preparation of the "Oropon" bates, which he patented in 1908.

There are many types of bating materials on the market today and while extravagant and unsubstantiated claims are made for some products, there is no doubt that the strength and the character of a bating material are of importance. But the proper selection of a bating material cannot be made merely on the basis of its so-called enzymatic strength; the nature of the beam-house processes employed at a given tannery, the kind of raw material used and the finished leather characteristics desired must all be considered in choosing the proper bate. The greater the knowledge of the tanner and leather chemist as to the function of bating, the more intelligently can they operate the process. For this reason, we are primarily concerned in this chapter with describing what is known of the principles rather than the details of the process. Wood and his contemporaries proved, as we have seen, that the active and necessary bate component is its proteolytic enzymes, but our present and yet limited knowledge of the manner in which the enzymes act upon the skin has resulted from more recent investigations. Before describing these studies it will be well to consider briefly the mechanics of bating.

Practically all light skins are bated prior to pickling and tanning, as are the matured hides used in the manufacture of side upper leather. The heavy hides which produce sole leather may or may not be bated before entering the vegetable tan liquors; if they are bated, the treatment is usually much less than that given light skins, and if not bated at all they often receive a chemical deliming. In the case of light skins, the limed and swollen skins are put into a paddle vat containing cool water, to which is usually added a small amount of a deliming acid, such as lactic or hydrochloric; the paddle is then revolved until the desired degree of deliming is attained. The extent of such preliminary deliming varies with individual processes and with personal preferences; it may consist of a mere surface deliming designed to remove any calcium carbonate precipitated on the skin's surfaces, or it may be considerably extended. At the completion of the

preliminary deliming, the skins are transferred to a paddle vat containing water at approximately 90° F (or whatever temperature is selected), to which the proper percentage of bating material is added. The selected temperature is maintained and the paddle is run continuously for the necessary time, after which the skins are considered "bated." The previously swollen skin is now flaccid; its grain has a smooth feel; it retains the impression of the fingers if squeezed; and it is permeable to air. The ammonium salts (sulfate or chloride) contained in the added bate have reacted with the lime which is combined with the incoming skin, greatly reducing its amount and thereby inducing the "fallen" condition noted and, at the same time, lowering the pH value of the skin to approximately 8.0 to 9.0—the optimum reaction for proteolytic enzymatic action. The time of bating treatment may vary from one to four hours in the case of calf skins; goat skins usually require a much longer time.

Any significant study of bating must be performed with skin itself, since investigations employing hide powder or other material bear no direct relation to the bating mechanism.

The various modern theories of bating have suggested the following possible effects of the proteolytic bate enzymes upon the skin. (a) Effect upon the skin collagen. (b) Effect upon skin elastin fibers. (c) Effect upon the reticular tissue of the skin. (d) Effect upon the degraded keratinous substances and scud, etc., present in hair follicles. (e) Effect upon the so-called "coagulable" proteins (albumins, globulins, etc.) which may remain in the limed skins.

Before discussing the five factors listed above let us consider some of the purely physical changes involved in bating. In considering the anatomy of skin in Chapter 2, we noted that it contains elastin fibers, involuntary muscle (the erector pili muscles), and voluntary muscle which is attached to its flesh surface, and which may not be completely removed when the skin is fleshed. In 1929, McLaughlin, Highberger, O'Flaherty and Moore[8] investigated the swelling and falling of these individual tissues as they passed through the several processes of the beam house. This was done by employing whole green salted calf skin, elastin fibers secured from calf ligamentum nuchae immediately after slaughter, involuntary muscle from the calf uterus and voluntary muscle from the thorax. These various salt-cured tissues were then given typical beam-house treatments of soaking, liming, bating and treatment with cold water after bating. (The last treatment was included to explain the fact that if bated skin is plunged into cold water its grain will become rough and will usually remain so in the finished leather.) The bating system employed was a solution containing 3.0 grams Oropon C per liter at a bating temperature of 95° F. Duplicate specimens were "bated" with lactic acid and with ammonium chloride in the absence of

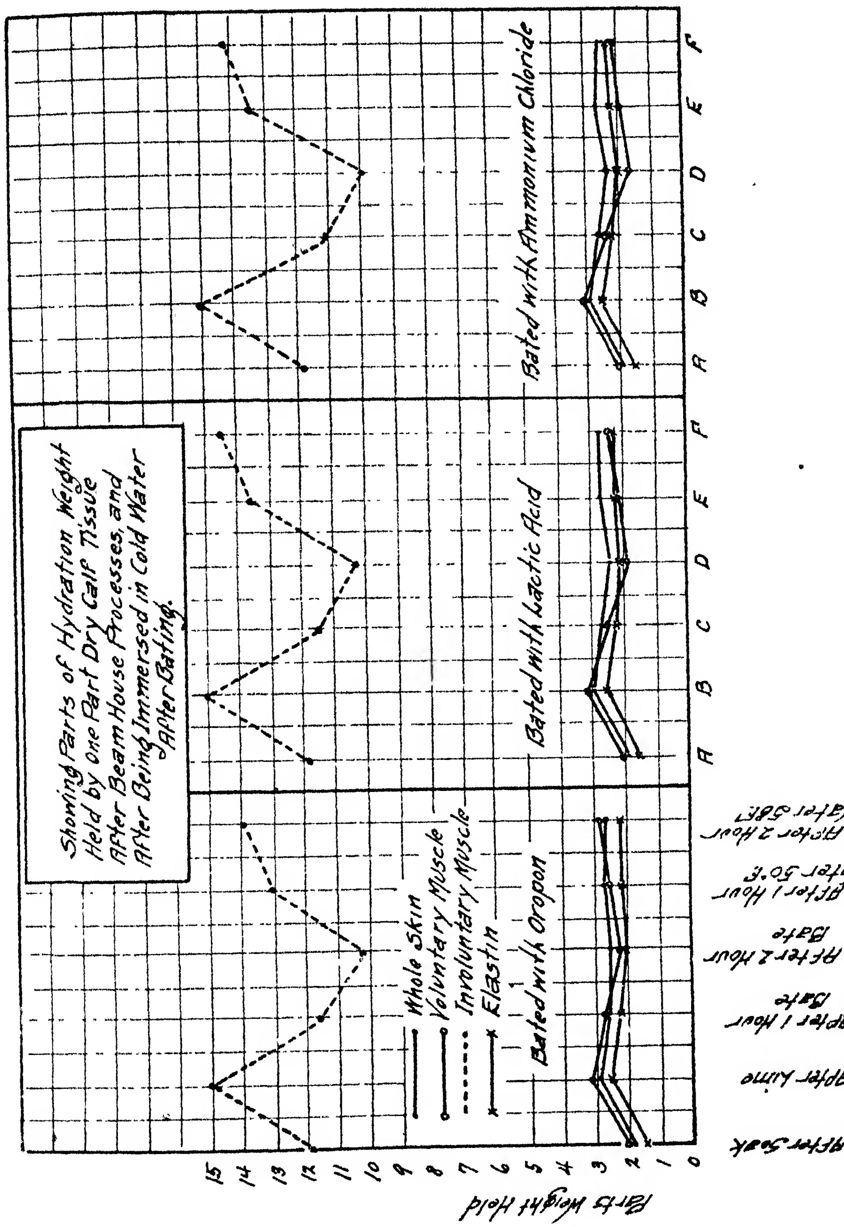

Figure 62

enzymes. The results are summarized in Figure 62, where the weight changes are expressed as parts of water or solution held by one part of dry material.

Figure 62 indicates the following. (1) The various tissues differ in swelling and falling values, involuntary muscle showing very much greater hydration throughout. (2) The weight of each soaked tissue is greatly increased during liming, but the soaked weight is regained after a two-hour bating treatment. (3) When the bated tissues are placed in water at 50° F they become hydrated and swollen, and this is particularly apparent in the case of the involuntary muscle. (4) All the physical changes noted are induced by a "bating" (deliming) treatment with either lactic acid or ammonium chloride, indicating that enzymes are not required to accomplish the physical changes noted; this confirms Merrill's[12] observation that the presence of enzymes is essentially without influence upon the falling of the whole skin during bating.

Effect of Bate Enzymes upon Skin Collagen. The principal enzyme present in most synthetic bates is trypsin. In 1923, Thomas and Seymour-Jones[19] studied its effect upon hide powder and found that considerable digestion occurs. The value of their experiments was questioned by Marriott,[10] in 1926; he correctly pointed out that hide powder is a denatured material. Marriott suggested that the liming process produces a degraded form of collagen, and that its digestion and removal is one of the functions of the bate enzymes. In 1927, Merrill and Fleming[11] made extensive studies of the digestion of collagen by trypsin, and concluded that the collagen of both fresh and limed skin may be digested by trypsin but that probably but little or no digestion of collagen occurs during practical bating operations. In 1934, Bergmann[2] discussed the digestion of collagen by trypsin; he stated that trypsin does not hydrolyze the proline nitrogen linkages in collagen, and that the same conclusion holds true in regard to the bating process when pancreatin is employed. He concluded that trypsin is able to hydrolyze only such peptide linkages as contain peptide hydrogen.

Effect upon Skin Elastin Fibers. In 1916, Rosenthal[17] suggested that the function of bating was essentially its removal of the skin elastin fibers. This conception was accepted by Krall,[7] Moeller,[13] Seymour-Jones[18] and particularly by Wilson,[22] who made very extensive microscopic studies of the effect of bating upon the elastin fibers. In 1922, Röhm and Haas[16] showed by microscopic means that neither manure nor synthetic bates appreciably affected the elastin fibers during normal bating; and their finding was confirmed by Marriott,[10] in 1926. Indeed, Wilson's original investigations indicated no appreciable digestion of elastin fibers under the conditions of practical bating; digestion occurred only when drastic bating methods, such as would not be employed in practice, were used. And Wilson later abandoned his contention as to the importance of the digestion of elastin.

Effect upon Reticular Tissue. In 1926, Turley[20] called attention to a tissue present in the corium major which, as he expressed it: "forms a sheath enveloping and protecting the bundles of connective tissue fibers." Turley

termed the tissue "areolar," but it has since become known as "reticular." This tissue has been extensively studied by Kaye,[6] employing steer hide; in 1936 she reported upon it as follows. "A tissue is present in skin which is composed of very fine fibers, binding together the coarser structures of the skin. It differs chemically from collagen and elastin, and structurally from the fibers composed of these. The tissue is very similar to the reticular tissue which is present in other parts of the animal body. It is the fibers of this tissue which bind together the collagen fibrils and fibers into bundles and cause the constrictions in the bundles when swollen with acids or alkalies. The tissue gives color reactions peculiar to proteins. It is digested by pepsin, but not readily by trypsin; it is attacked by the action of bacteria. It is very resistant to chemical reagents and withstands much longer than collagen the action of strong acids and alkalies." In 1939, Roddy and O'Flaherty[14] stated that the tissue composing the walls which are between fat cells consists essentially of reticulin, and that this tissue is comparable to the reticular tissue located between the collagen fiber bundles, and hence would serve as experimental material. They therefore extracted the fat from adipose tissue cut from the kidney area of the flesh side of steer hide. The reticular tissue remaining after the fat was extracted was then studied with the following results. The tissue seemed to be a conjugation of proteins giving tests for albumins, globulins and mucoids; these three compose a small fraction of the total tissue. The large fraction of the tissue was of a different protein type; it was digested by pepsin but not trypsin.

In their bating studies of 1929, McLaughlin, Highberger, O'Flaherty and Moore[8] suggested, on the basis of microscopic examination of skin before and after bating, that one of the important functions of bate enzymes was their digestion of the reticular tissue associated with the collagen fiber bundles. But in view of the finding of both Kaye and Roddy and O'Flaherty that reticular tissue is not digested by trypsin, this conclusion would now seem to be unwarranted. The phenomenon which these former workers observed microscopically may possibly be best explained as follows. Rupture of reticulin fibers is probably a function of lime swelling; but since these fibers appear to be coated with an albumin-globulin fraction as noted above, they would not be stained so clearly as would be the case after the bate enzyme had digested away the coagulable protein coating. In other words, the ruptured condition found probably existed before the skin entered the bate but was not made visible by staining until the reticular fibers had been cleansed of their coating. Turley,[21] on the other hand. has experienced no difficulty in staining and demonstrating reticular tissue out of lime. He finds it to be unaffected by the bate enzymes.

Effect upon Keratinous and Other Degradation Products. In 1919, Gautrelet and Thuau stated that the main function of enzymatic bates is

their hydrolyzing action upon the keratinous material remaining in the limed skin. In 1926, Wilson and Merrill[23] suggested that the most important function of bate enzymes is probably their hydrolysis of the degraded keratinous matters present in the skin thermostat "layer," which, if not removed, will be precipitated by the acid tan liquors and will adversely affect the appearance of the finished leather grain. They therefore prepared an experimental material which they termed "keratose" by digesting clean calf hair with sodium hydroxide. They then studied the action of both pancreatin and commercial bates upon keratose and found a very con-

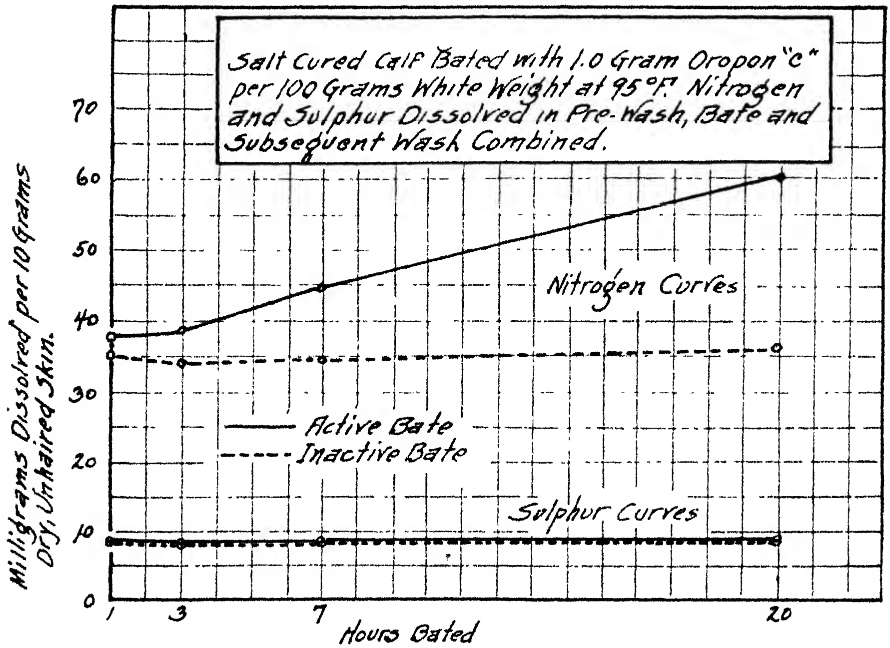

Figure 63

siderable digestion to occur in the case of both enzyme sources. We shall not enlarge upon the very extensive and interesting experimental studies with keratose by these authors, since it was shortly thereafter shown that while degraded keratinous matters are removed in bating, their removal is not an enzymatic function.

In 1929, McLaughlin, Highberger, O'Flaherty and Moore[8] made a careful study of the amount of keratinous material removed in the bating of both green salted calf and sun-dried (unsalted) goat skins. The soaked skins were limed without the addition of sulfide or other accelerators, were fleshed, unhaired and scudded, and were then given the following bating treatments.

Portions of scudded skin were paddled in distilled water at 68° F for 10 minutes, when the wash water was removed and was replaced with fresh. Thus three waters were given over a washing period of 30 minutes. The washings were then mixed, filtered, and analyzed for sulfur and for nitrogen.

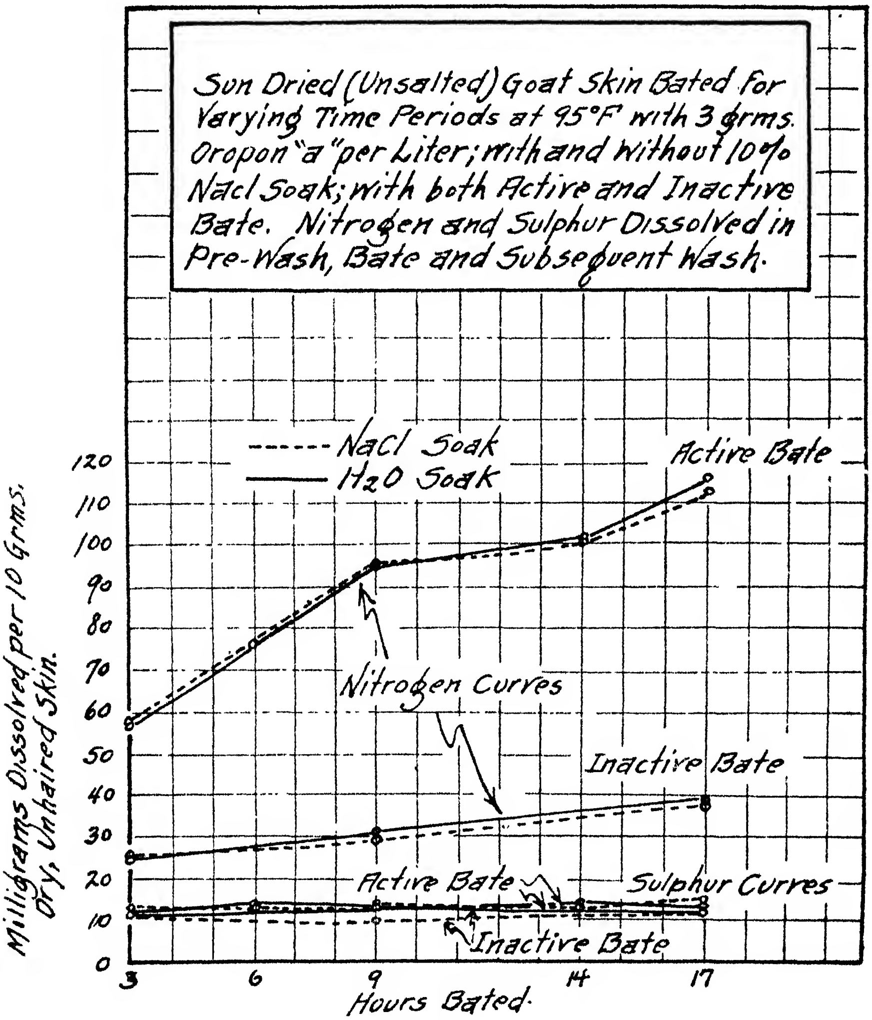

Figure 64

The object of the preliminary washing was to remove surface and other easily removable degradation products, and thus to bring the skin into a uniform condition prior to bating. The washed skin was then bated for the time periods and at the temperatures shown, using 1.0 per cent Oropon C

for calf and 3.0 per cent Oropon A for goat skin, based upon white weight. Duplicate experiments were run in all cases; in one experiment the bating material possessed the normal enzymatic activity, but in the duplicate experiment the enzymes of the bate liquor had been completely inactivated by heating it in a water bath for 50 minutes at 88° C. At the end of the bating treatment, the skin was again washed in three changes of distilled water, in the same manner as described for the pre-bating wash. The after-bating wash waters were combined, filtered, and analyzed. When the dissolved nitrogen and sulfur values found for the pre-wash, the bate liquor and the after-wash were added together and were plotted against hours of bating, the values shown in Figures 63 and 64 were found.

It will be noted that the dissolved sulfur values shown in the two figures are practically identical, whether an active or inactive bate is employed. Since sulfur is a distinguishing characteristic of keratinous matters, it is obvious that if the function of the bate enzyme were to hydrolyze and remove keratinous degradation products from the skin, more sulfur should have been present in the active enzyme bating solutions; but such is not the case. In other words, the removal of keratose is not an enzyme function.

Effect upon Coagulable Proteins Present in Skin. The suggestion that a prime function of bating is the effect of the bate enzymes upon the interfibrillary protein matter of the limed skin was first made by Eitner,[3] in 1911. Similar suggestions were made by Marriott[9] in 1921, Röhm and Haas[16] in 1922, and by Turley[20] in 1926. None of these authors attempted to confirm the suggestion experimentally, but in 1924 Kaye and Jordan-Lloyd,[5] in their studies of collagen swelling, demonstrated the presence of interfibrillary material and showed that it was digestible by trypsin. In their bating investigations already referred to, McLaughlin, Highberger, O'Flaherty and Moore experimentally studied this phase of bating and concluded that the removal of interfibrillary matter is probably the principal function of the bate enzymes. Their study was made in two ways. Laboratory experiments, conducted according to the methods already described, were run with dry, sun-cured (unsalted) goat skin, since such material retains all of its original interfibrillary material which, in drying, becomes hardened. Duplicate specimens were soaked in plain water and also in 10 per cent sodium chloride solutions (followed by plain water) to disperse and remove part of the interfibrillary nitrogen. Analysis of the various soak solutions at completion of soaking showed, as would be expected, a very much greater amount of coagulable nitrogen removed by the salt soak. All skin specimens were then limed, unhaired and scudded, after which they were bated with increasing concentrations of U.S.P. pancreatin, ranging from 0.01 to 0.10 gram per liter, sufficient ammonium chloride being added to each bate solution to maintain a constant pH value of 8.3-8.5 throughout the 16-hour

bating period at 95° F. When the nitrogen dissolved in soak, lime, pre-wash, bate and after-wash were added together the total was uniformly much greater in all instances in the case of the salt soak, that is, as a function of bate enzyme concentration, as shown in Figure 65.

The difference in value of the two nitrogen curves of the figure is very approximately accounted for by the greater amount of coagulable nitrogen which was dissolved from the skin by the salt soak. And it will be noted

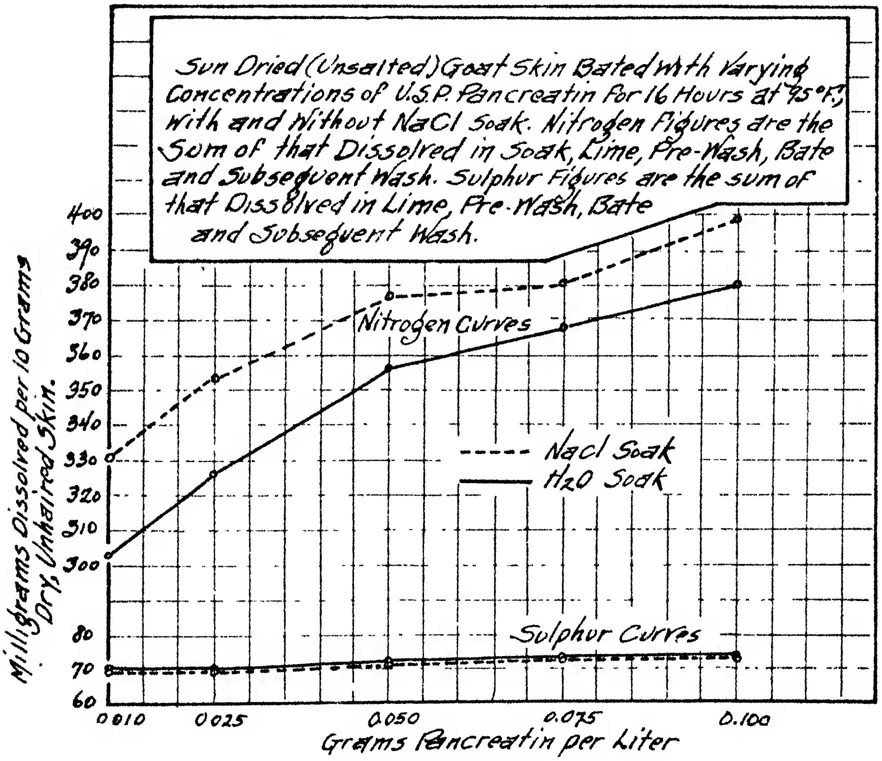

Figure 65

that as much total beam-house nitrogen was dissolved in 16 hours by 0.05 gram pancreatin in the case of the salt soak as with double the enzyme concentration in the case of the water soak. Or, when the goat skin was bated with 3.0 grams Oropon A per liter, the same amount of total dissolved nitrogen was found at the end of 9 hours' bating of the salt-soaked skin as was removed by 17 hours' bating of the water-soaked. These results led to the second experimental method employed; that is, to large-scale tannery tests. Comparative packs of sun-cured goat skins were processed according to the

regular tannery processes of that time: long soak, long liming with much added sulfide, and long, drastic bating. When no modifications of such beam-house processes were made, skins soaked in 10 per cent sodium chloride solutions produced poor quality, thin, raggy leather compared with those soaked in plain water. But when the experiments were repeated and the liming and bating were properly shortened on the basis of careful examination of the skins being processed, it was found that salt-soaked skins required 30 per cent less liming and 33 per cent less bating time. When these two processes were thus modified, the salt-soaked skins produced leather which was superior in fullness, feel, etc. to that which had received the normal and the then usual beam-house treatments.

These investigators concluded that the leather results noted were to be explained as follows. Skin fibers which are covered with coagulable and dehydrated protein material require more drastic soaking, liming and bating than if less of such material is present. But if such coating material is partially removed in the soak, a somewhat "naked" fiber enters the lime and the bate. And such naked fibers do not need- and, indeed, cannot stand- - the drastic liming and bating treatments necessary for fibers which are encased in dehydrated interfibrillary material.

H. Anderson[1] has recently studied the removal by trypsin of the interfibrillary protein remaining in delimed goat skin. He finds that the enzyme removes a large proportion of such material, but states that its removal appears to have no effect upon the characteristics of the finished leather. The latter statement cannot of course be taken too seriously, since Anderson based this conclusion on the leather produced from only two skins. Tanning experience has long shown that conclusions involving the "feel" and other characteristics of leather may not be safely drawn unless based upon the examination of, say, several hundred skins.

In attempting to sum up our present knowledge of the bating process, it is necessary to divide the effects of bating into two classes. In the first are to be grouped all those changes in the skin which may be induced by the action of solutions of deliming acids or salts and for which proteolytic enzymes are not required. In the second class are included all changes which require the presence of proteolytic enzymes. The only necessary enzymatic action which appears to have been reasonably well established to date is the dispersing effect of proteolytic enzymes upon the interfibrillary protein present in the skin as it reaches the bate paddle. There would seem to be no reasonable doubt that large-scale tannery experience has proved that the extent of *enzymatic action* required is entirely dependent upon the nature of the soaking and liming which has preceded the bate. If, for example, a large proportion of interfibrillary protein has been removed in the

soak by the addition of sodium chloride or other dispersing salts, or of sodium polysulfide, the bating treatment must be reduced, or inferior leather will result. Conversely, if the bulk of interfibrillary matters has been left in the skin, a more drastic bating is required. This does not necessarily mean that there are not other important enzymatic functions which the future will disclose. But we would suggest that the value of future studies will depend upon the extent to which laboratory investigations are correlated with tannery experiments under actual operating conditions.

We have left untouched the subject of the analytical evaluation of bating materials. This information is readily available in the numerous publications on the subject.[4]

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Chapter 11

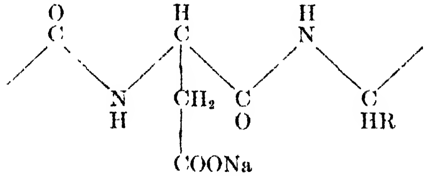
Pickling

In Volume I of the second edition of this monograph, the pickling operation was little more than described, since up to 1928 the process had not been systematically investigated. Our understanding of the process at that time rested upon the general studies of Procter,[10] Wilson,[11] and Loeb.[6,24] These investigations dealt with the swelling and the acid- and base-binding of gelatin, and from them the Procter-Wilson theory of swelling was derived. This theory, which applied the Donnan membrane equilibrium to swelling, has been adequately described and discussed in Volume I.

In Chapter 4, the electrolyte-fixation capacity of fibrous proteins has been discussed in detail. As the acid- and salt-fixation power of collagen is of great importance in the pickling process, the work described in that chapter may be considered the foundation of pickling.

The term "pickling" may be defined as the process of treating bated hides and skins with mixed solutions of acid and salt. The process is of importance for two different reasons, namely, the conditioning of hides and skins for subsequent mineral tannage or the preservation action necessary for their long storage.

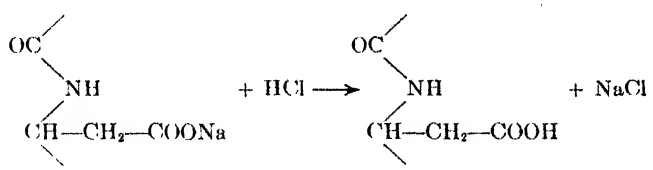
During contact with the alkaline unhairing bath, the skin proteins have become swollen and alkali-fixation has occurred corresponding to that normally obtaining at pH 12.5 to 13.0. The sodium and calcium ions are bound to the proteins thus:



In addition to the cation fixed, unbound alkali is diffused throughout the skin tissue. This however, is usually removed in the subsequent bating and washing operations, and can be largely discounted. During the bating process, the pH value of the skin tissue is decreased from 12.5 to 7.5 to 9.0, depending upon the *modus operandi* of the operation. In consequence of the bating operation, a portion of the bound alkali is removed and only that

alkali remains which corresponds to that normally obtaining at the particular pH value of the bating.

During the early period of pickling, the acid acts first upon the protein (collagen)-alkali compound, converting it into isoionic protein, *i.e.*, collagen which is combined equally with hydrogen and hydroxylione:



and secondly, upon the isoionic collagen, converting it to a collagen-acid compound, $^{+}H_3N—R—COO^{-} + HCl \rightarrow Cl^{-}\ ^{+}H_3N—R—COOH$. The acid continues to bind with the skin proteins until equilibrium has been established. The acid fixed during any pickling operation is that obtaining for the equilibrium pH value of the pickle solution.

Most pickle solutions used have an equilibrium pH value ranging from 1.5 to 2.5. If acid alone were employed, the hide or skin would swell beyond that which is desired. If salt is present, however, the amount or degree of swelling can be controlled. It is in this particular pH range that maximum acid swelling obtains and therefore sufficient salt (NaCl) must be added so that only the desired degree of swelling occurs.

In addition to the bound acid, the skin contains or holds within itself a certain amount of acid-salt solution and for this reason both the bound and free acid in the skin must be taken into account in the subsequent mineral tannage.

In the period 1920 to 1930 but little real study was made of this very important process and any advancement noted was in the field of practice. However, in 1930, Küntzel, Buchheimer and Priesentanz[5] investigated the pickling action of various acids upon specially prepared collagen. This work was the first definite study dealing with skin collagen, and from that viewpoint deserves special mention in any treatise dealing with the pickling process.

For their investigation, Küntzel and collaborators used a raw collagen material prepared after the manner described by Buchheimer, namely, cow hide freshly slaughtered, the flesh and epidermal layers being removed by splitting. The skin thus prepared was then air-dried and cut into strips and was ready for use. This type of prepared skin was first used by McLaughlin and Theis in 1921. Küntzel used 8 different proportions of hydrochloric acid varying from 0 to 2 gram equivalents per 1000 grams of dry collagen, while the salt varied from 0 to 60 gram equivalents. His method was to treat 1 gram of the collagen with 10 ml of the particular pickle solution for 24 hours. After such treatment, the collagen was removed,

drained and weighed. The acid absorption was determined by titration of the residual pickle liquor. Küntzel found that as the amount of given acid increased, the amount of acid bound gradually increased to a maximum of 0.7 milliequivalent per gram of dry collagen—this value resulting from the employment of 0.8 milliequivalent of acid per gram of collagen. In the presence of salt, the maximum acid fixation is increased slightly. Küntzel pointed out that salt fixation is so slight as to be entirely negligible. He showed that the degree of pickling as gauged by the suppression of swelling is not dependent on the equivalent ratio of acid to salt.

Küntzel and Priesentanz, in a further study of the mode of action of various acids, found that at any given acid concentration, the absorption of weak acids is less than that with strong acids, but that at a given initial pH value the absorption of weak acids is greater. Table 116 illustrates this point.

Table 116

	HCl	H_2SO_4	$H \cdot COOH$	$C_3H_6O_3$	CH_3COOH
Milliequivalent acid used	0.80	0.80	0.80	0.80	0.80
Milliequivalent acid absorbed	0.67	0.70	0.32	0.29	0.19
Milliequivalent acid used	2.00	2.00	2.00	2.00	2.00
Milliequivalent acid absorbed	0.70	0.70	0.44	0.40	0.30
Initial pH 2.4	0.035		0.30		

From 1930 to 1942, Theis *et al.* made a comprehensive and systematic study of the pickling operation. This work will be discussed under appropriate headings throughout this chapter.

In any discussion of the principles underlying the pickling process, there are a number of factors that must be considered, *i.e.*, (a) acid concentration; (b) kind of acid; (c) neutral salt concentration; (d) volume of pickle solution in relation to skin; (e) hydrolysis and peptization factors; (f) temperature effects; (g) deliming effects; (h) preserving effects; (i) effect upon skin "grain"; and (j) effect upon subsequent tannage.

Theis and Goetz[14] in their systematic study of pickling made use of the following technique. Salt-cured steer hide was soaked for 24 hours at 20° C. The soaked and fleshed stock was limed in a straight lime liquor, using 10 per cent excess solid calcium hydrate, for 5 days at 20° C. After unhairing and further fleshing, the hide was cut into small cubes and washed thoroughly in running water for several hours. The washing was followed by bating at 37.5° C, using 1.0 per cent Oropon until the proper depletion of swelling and neutralization had occurred. The bated cubes were then washed thoroughly and surface-dried. The samples for the individual pickle solutions weighed 45 grams and measured 41 ± 1 ml in volume. The weighed samples were placed in 450 ml of pickle solution, containing various amounts of salt and acid, and agitated at hourly intervals for 24 hours at 20° C.

After such treatment, the hide cubes were again surface-dried, weighed,

Table 117. 12.7 Millimols HCl per 100 grams Dry Bated Stock.
(0.35 gram hydrochloric acid (40%) per 100 grams bated stock, 70% moisture.)

NaCl used		pH of solution		Per cent gain		M mols HCl consumed per 100 gms dry stock	Per cent original HCl consumed	Milligrams nitrogen per 100 gms dry stock	Gms NaCl absorbed 100 gms bated stock
Gms per 100 gms bated stock	Millimols per liter	Before	After	Wt	Vol.				
(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)	(j)
0.	0.	2.63	4.98	2.0	2.0	11.9	93.6	132.	0.
2.5	42.7	2.42	5 64	4.0	2.0	12.3	96.9	132.	−0.5
5.0	85.4	2.42	5.84	0.0	0.0	12.0	94 5	140.	−0.25
7.5	128.1	2.42	6.10	4.0	0.0	12.2	96.0	150.	
10.0	170.8	2.42	6.21	2.5	2.0	12.0	94.5	160.	−0.07
15.0	256.2	2.40	6.43	4.9	4.9	12.4	97.6	180.	0.85
20.0	341.6	2.40	6.21	4.8	2.0	12.4	97.6	181.	1.35
30.0	512.4	2.37	6.21	2.5	2.0	12.0	94.5	170.	2.10
45.0	768.6	2.37	6 52	7.5	7.2	12.4	97.6		3.40

Table 118. 25.4 Millimols HCl per 100 grams Dry Bated Stock.
(0.70 gram hydrochloric acid (40%) per 100 grams bated stock, 70% moisture.)

Salt used		pH of solution		Per cent gain		M mols HCl consumed per 100 gms dry stock	Per cent original HCl consumed	Milligrams nitrogen per10 gms dry stock	Gms NaCl absorbed 100 gms bated stock
Gms per 100 gms bated stock	Millimols per liter	Before	After	Wt.	Vol				
(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)	(j)
0.	0.	2.13	3.26	12.5	17.0	22.05	86.7	89.	
2.5	48.7	2.12	3.62	6.5	12.0	23.42	92.1	120.	−0.5
5.0	85.4	2.10	3.82	5.5	7.2	23.42	92.1	130.	−0.7
7.5	128.1	2.08	4.03	4.9	4.2	24.00	94.5	150.	0.13
10.0	170.8	2.08	4.35	3.5	2.2	24.00	94.5	151.	0.35
15.0	256.2	2.08	4.34	1.5	0.0	24.00	94.5	140.	0.65
20.0	341.6	2.07	4.85	1.0	−2.5	24.40	96.0	128.	0.78
30.0	512.4	2.07	4.74	0.0	0.0	24.40	96.0	140.	2.34
45.0	768.6	2.01	4.61	0.5	0.0	24.40	96.0	140.	3.20

Table 119. 63.5 Millimols HCl per 100 grams Dry Bated Stock.
(1.75 grams hydrochloric acid (40%) per 100 grams bated stock, 70% moisture.)

Salt used		pH of solution		Per cent gain		M mols HCl consumed per 100 gms dry stock	Per cent original HCl consumed	Milligrams nitrogen per 100 gms dry stock	Gms NaCl absorbed 100 gms bated stock
Gms per 100 gms bated stock	Millimols per liter	Before	After	Wt.	Vol.				
(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)	(j)
0.0	0.0	1.79	2.25	28.0	29.2	44.7	70.4	174.	0.0
2.5	42.7	1.79	2.51	20.5	22.0	51.4	81.0	161.	−0.5
5.0	85.4	1.79	2.66	17.7	19.5	52.8	83.2	162.	−0.34
7.5	128.1	1.77	2.77	16.4	17.0	55.2	86.8	130.	
10.0	170.8	1.77	2.77	15.0	17.0	56.0	88.2	170.	0.12
15.0	256.2	1.76	2.83	8.0	7.0	56.0	88.2	160.	0.50
20.0	341.6	1.74	2.88	−1.0	0.0	56.6	89 1	152.	0.78
30.0	512.4	1.74	3 09	−3.0	−2.5	58.2	91.5	132.	2.00
45.0	768.6	1.72	3 02	−2.5	−7.3	58.8	92 6	132.	3.10

Table 120. 127.0 Millimols HCl per 100 grams Dry Bated Stock.
(3.50 grams hydrochloric acid (40%) per 100 grams bated stock, 70% moisture.)

NaCl used		pH of solution		Per cent gain		M mols HCl consumed per 100 gms dry stock	Per cent original HCl consumed	Milligrams nitrogen per 100 gms dry stock	Gms NaCl absorbed 100 gms bated stock
Gms per 100 gms bated stock	Millimols per liter	Before	After	Wt.	Vol				
(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)	(j)
0.0	0.0	1.47	1.88	30.8	34.0	75.4	59.4	194.	0.0
2.5	42.7	1.48	1.95	27.0	28.2	85.6	67.5	187.	
5.0	85.4	1.48	2.00	22.5	24.3	90.7	71.5	158.	−0.4
7.5	128.1	1.48	2 00			90.7	71.5	139.	−0.2
10.0	170.8	1.48	2.08	19.3	22.0	95.0	74.8	159.	0.0
15.0	256.2	1.47	2.11	17.0	17.0	98.2	77.3	150.	
20.0	341.6	1.45	2.15	13.0	12.1	100.0	78.8	161	0.65
30.0	512.4	1.45	2.15	−3.3	−7.3	101.5	80.0	152.	1.25
45 0	768.6	1.43	2 15	−3 9	−7.3	104.0	82 0	132.	2.50

Table 121. 192.0 Millimols HCl per 100 grams Dry Bated Stock.
(5.25 grams hydrochloric acid (40%) per 100 grams bated stock, 70% moisture.)

NaCl used		pH of solution		Per cent gain		M mols HCl consumed per 100 gms dry stock	Per cent original HCl consumed	Milligrams nitrogen per 100 gms dry stock	Gms NaCl absorbed 100 gms bated stock
Gms per 100 gms bated stock	Millimols per liter	Before	After	Wt	Vol.				
(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)	(j)
0.0	0.0	1.28	1.57	32.5	36.5	88 5	46 2	213.	0.0
2.5	42.7	1.32	1.60	27.5	31.5	97.2	50.6	196.	−0.48
5.0	85.4	1.32	1.62	23.0	24.5	101.0	52.5	196.	−0.22
7.5	128.1	1 30	1 62	20.5	22.0	102.5	53.3	184.	−0.02
10.0	170.8	1.28	1.62	19.0	19.5	103.2	53.8	188.	0.02
15.0	256.2	1.28	1.62	17.0	17.0	106.2	55.3	209.	0.50
20.0	341 6	1.26	1.59	12.8	12.5	105.5	54.8	199.	1.20
30.0	512 4	1.25	1.59	−0.7	−2.5	105.2	54.6	189.	1.30
45.0	768.6	1 21	1.59	−1.0	−2.5	105.2	54.6	200.	3.00

Table 122. 254.0 Millimols HCl per 100 grams Dry Bated Stock.
(7.0 grams Hydrochloric acid (40%) per 100 grams bated stock, 70% moisture.)

NaCl used		pH of solution		Per cent gain		M mols HCl consumed per 100 gms dry stock	Per cent original HCl consumed	Milligrams nitrogen per 100 gms dry stock	Gms NaCl absorbed 100 gms bated stock
Gms per 100 gms bated stock	Millimols per liter	Before	After	Wt	Vol.				
(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)	(j)
0.0	0.0	1.15	1.42	29.5	29.2	104.2	41.0	271.	0.0
2.5	42.7	1 21	1.40	25.5	24.5	106.5	41.8	215.	−0.4
5.0	85.4	1.16	1.38	22.5	22.0	108.9	42.8	226.	
7.5	128.1	1.16	1.38	20.0	20.5	108.9	42.8	225.	−0.14
10.0	170.8	1.16	1.40	18.5	19.5	109.5	43.2	217.	0.13
15.0	256.2	1.16	1.40	17.0	17.0	111.0	43.6	208.	0.71
20.0	341.6	1.13	1.36	13.3	12.0	111.1	43.6	219.	1.05
30.0	512.4	1.13	1.36	2.3	2.0	111.1	43.6	201.	2.10
45.0	768 6	1.11	1.33	−0.5	−2.5	111.1	43.6	212.	3.50

and the change in volume determined. The residual pickle solutions were analyzed for acid, salt, nitrogen and pH value. Samples of the original

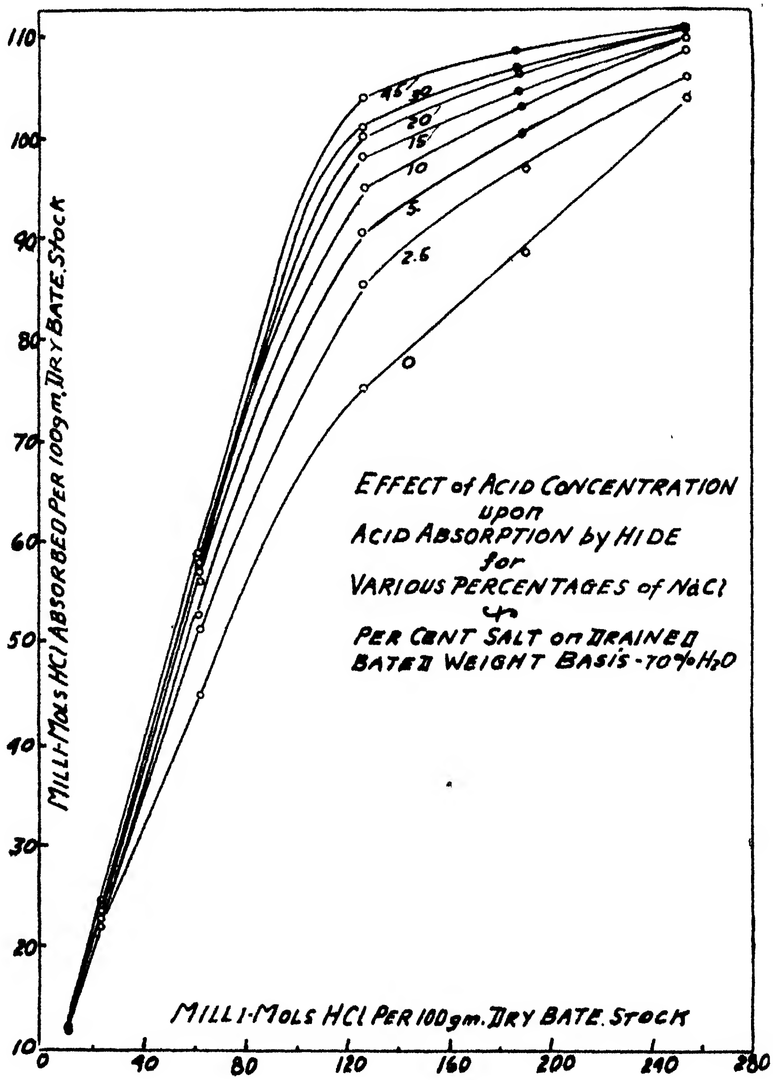


Figure 66

bated stock showed a calcium oxide content equivalent to 8.5 millimols of hydrochloric acid per 100 grams of dry stock. The moisture content of the bated cubes was approximately 70 per cent.

The Hydrochloric Acid-Salt System

For this investigation, Theis and Goetz[14] varied the hydrochloric acid concentration between 12.7 and 254 millimols and the sodium chloride

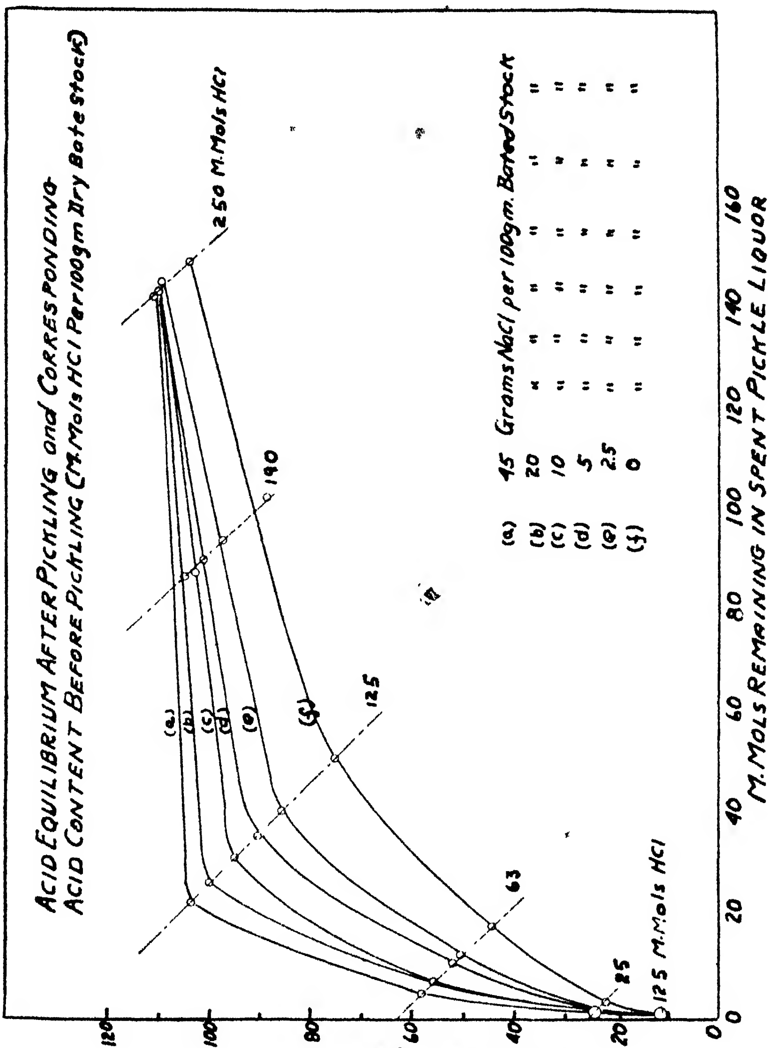


Figure 67

between 0 and 768.6 millimols per 100 grams bated stock. The data obtained are shown in detail in Tables 117 to 122 and in Figures 66 to 72.

Tables 117 to 122, columns g and h, and Figures 66, 67, 68 and 69 definitely show that the amount of hydrochloric acid absorbed per 100 grams

of dry bated skin increases with increasing concentrations of salt. With increasing concentration of acid the effect of salt is of lesser degree. The salt effect is shown more clearly in Figure 67 when the millimols of residual

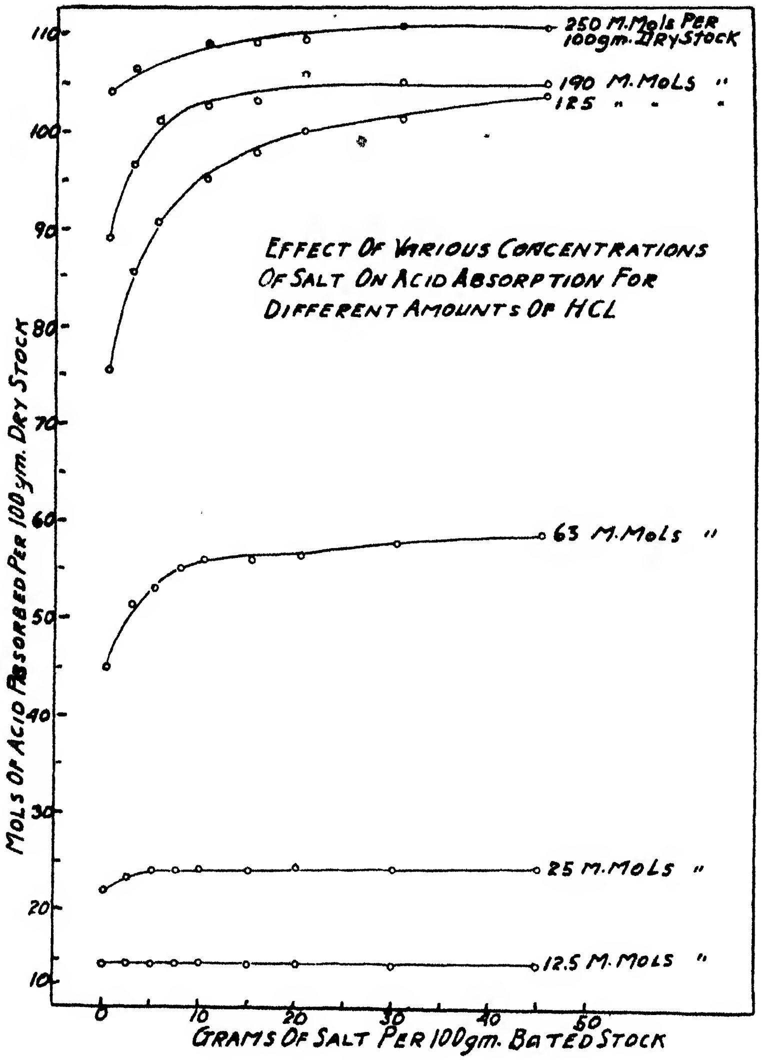


Figure 68

acid are plotted versus the millimols of acid absorbed, resulting in typical adsorption curves. If the amount of salt used is plotted against the acid absorbed, curves are obtained as shown in Figure 68. From these data, it

is seen that the salt effect upon the individual acid curves is greatest for the 125-millimol acid curve. The slopes of the several curves indicate that acid absorption by the skin is greatest between 0 and 20 per cent salt. Whereas above 20 per cent the salt effect upon acid absorption for any concentration of acid is but slight. Figure 69 shows the effect of the initial concentration of acid upon the amount of acid absorbed by the skin. These curves again

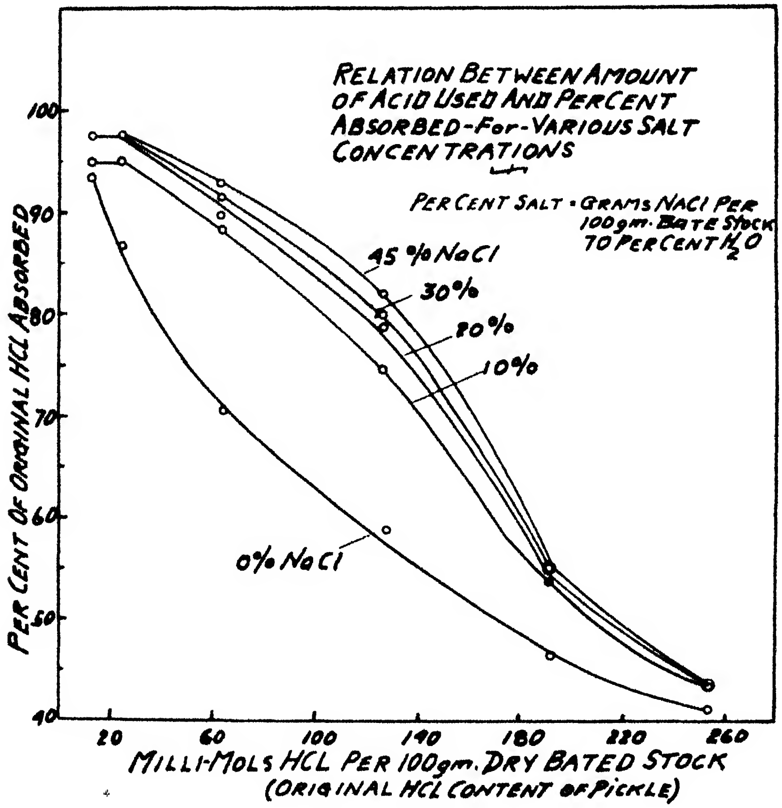


Figure 69

illustrate the effect of salt upon acid-absorption and definitely show that the percentage of initial acid absorbed by skin is almost inversely proportional to the amount of original acid used.

In order to determine swelling, Theis and Goetz measured both the weight gain and the volume change resulting from treatment with the various pickle solutions. The data are given in columns e and f of Tables 117 to 122 and in Figure 70.

Figure 70 shows in graphical form the effect of the various acid-salt

systems upon swelling during the pickling operation. It is of interest to note that for any given acid concentration there is a definite depression in swelling caused by the added salt. This depression of swelling is much more pro-

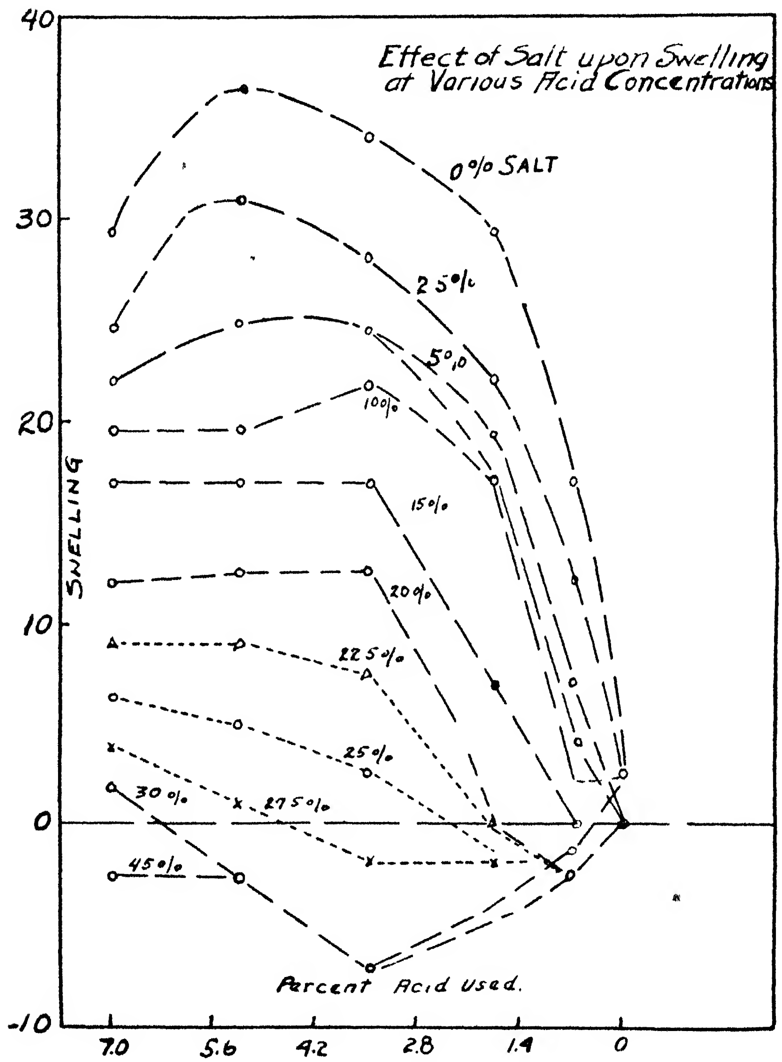


Figure 70

nounced in the region where acid solutions containing no salt gave a maximum swelling value. At values between 10 and 22.5 per cent salt, increasing amounts of acid (above 3.5 per cent) had but little further effect upon the

swelling of the skin. From Figure 70 it can readily be seen that between 20 and 27.5 per cent salt there is no rise to a maximum swelling value at the

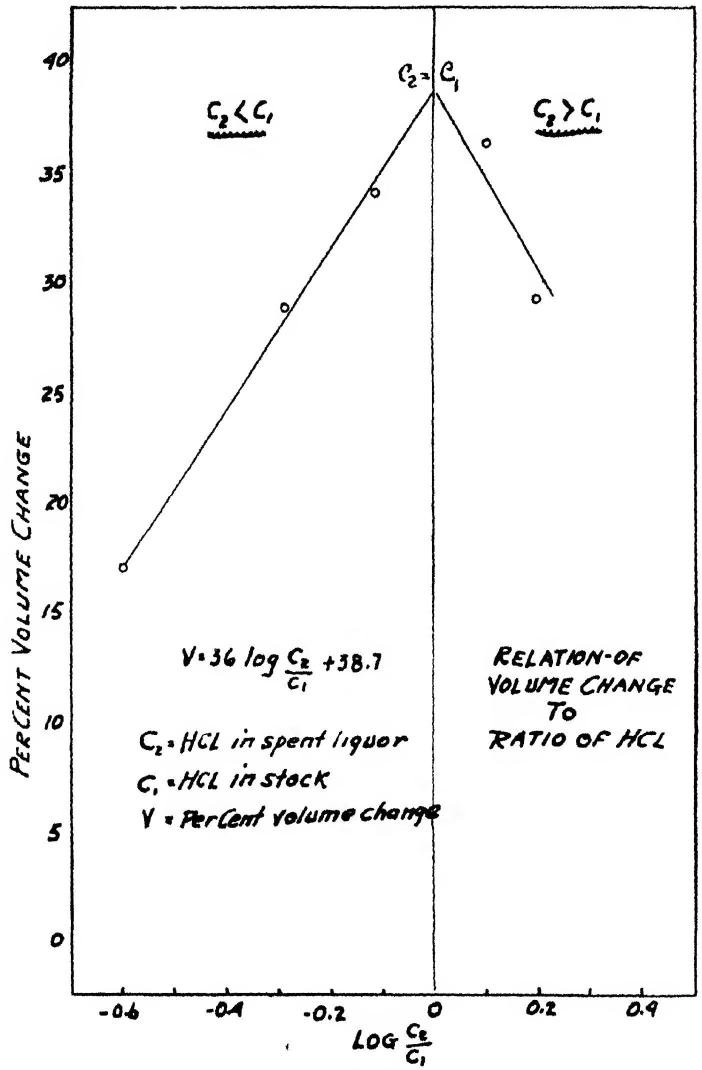


Figure 71

higher concentrations of acid. For the lower concentrations of acid, the swelling curves decrease sharply with decreasing amounts of acid. For the 30 and 45 per cent salt curves, it will be noted that over the whole range

of acid concentration there was no swelling, but in fact a negative swelling effect. This negative swelling is more pronounced at 3.5 per cent acid, below which point the negative swelling is less, and at 0.35 per cent the high

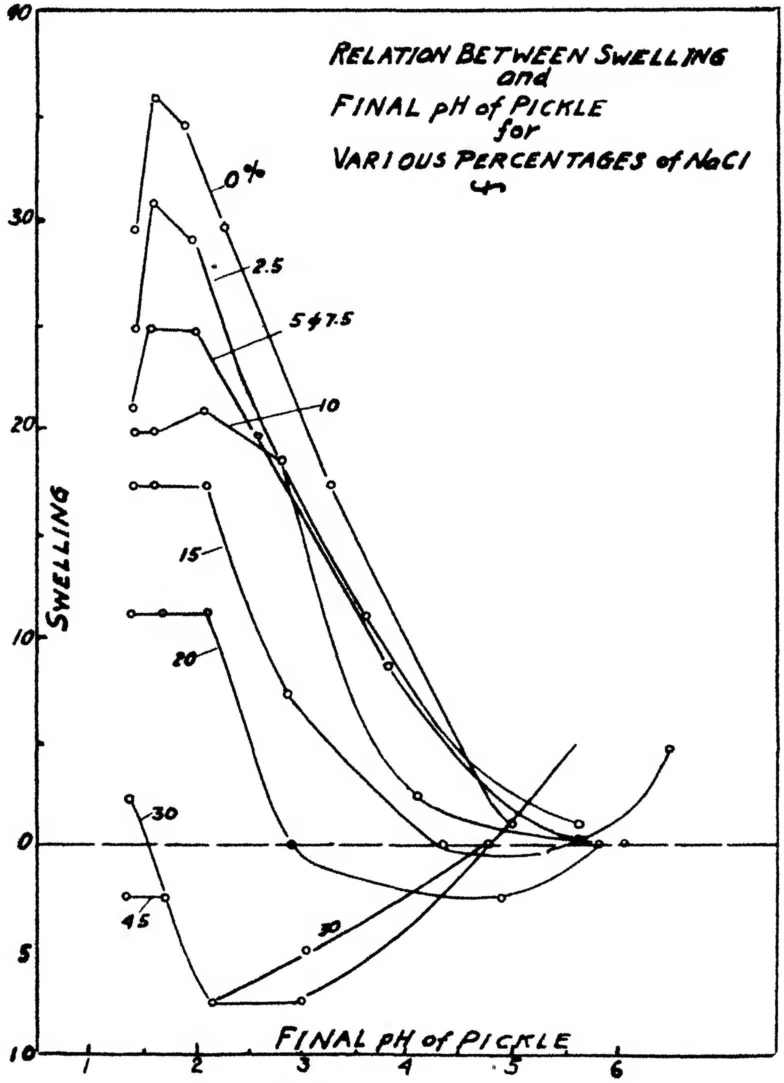


Figure 72

concentrations of salt cause a slight positive swelling. This reciprocal effect of acid and salt with purified collagen was also found by Küntzel and Priesentanz.

Theis and Goetz go on to point out from the data given in Figure 70 that the change of slope of all of the swelling-salt curves occurs in the vicinity of 3.5 per cent acid, or 127 millimols HCl per 100 grams dry bated skin. The data also show that at this acid concentration salt has its greatest effect upon acid absorption. This fact was attributed to the greatest excess of retained diffusible ions. According to Procter and Wilson, it is neither the concentration of the anion of the protein nor that of the ionized protein salt which is a measure of the swelling force, but the excess concentration of diffusible ions of the protein over that of the external phase. This excess in concentration in dilute solutions increases almost directly with increasing concentrations of acid; it approaches a maximum as the formation of the protein chloride nears completion, and then decreases as the concentration of the external phase increases. Therefore, since this amount of excess concentration is a function of both the amount of acid absorbed and the amount existing in the external phase, there should be a definite relation between these two values and the amount of swelling produced. Theis and Goetz show a correlation between acid absorbed and swelling by plotting the log of the ratio of acid unabsorbed to that absorbed by the skin versus swelling. These data are given in Table 123 and in Figure 71.

Table 123. Relation between Swelling and Ratio of Acid in Spent Liquor and in Hide.

M mols HCl in stock (C_1)	M mols HCl in spent liquor (C_2)	$\frac{C_2}{C_1}$	Log $\frac{C_2}{C_1}$	Per cent volume change
3.4	0.8	0.235	−0.628	2
13.55	3.4	0.251	−0.600	17
36.2	18.9	0.522	−0.283	29
66.9	51.5	0.770	−0.144	34
80.0	103.4	1.292	0.122	36.5
96.7	150.00	1.550	0.190	29.2

Whenever $C_2 < C_1$ there is an increase in swelling, and whenever $C_2 > C_1$ there is a decrease. Furthermore, the point of maximum swelling occurs when $C_2 = C_1$, *i.e.*, at this value there is the greatest excess of diffusible ions within the skin Any further addition of acid, making $C_2 > C_1$, will obviously cause a decrease in the amount of excess ions and consequently result in less swelling. Swelling in this case may be said to be due to the flow of water from the less concentrated to the more concentrated solution within the skin which, thermodynamically speaking, causes an increase in the entropy of the system; in other words, the partial mole free energy of the ions within the skin and in the external solution must be the same. Thus the amount of swelling taking place during pickling is primarily dependent upon the amount of excess diffusible ions within the skin.

On this basis it is quite evident that by adding a small amount of salt to the skin-acid system the excess of ions will be increased, and a condition

exists similar to that for high concentrations of acid. Upon further addition of salt this effect is accentuated in that there is now a flow of water from the less concentrated solution within the skin to the more concentrated external solution, and thus a decrease in swelling results.*

Since the excess of diffusible ions is the direct cause for the difference of potential between the external and internal solutions, salt will have its greatest effect on repression of swelling when this condition exists and when the difference of potential is greatest, as is shown in Figure 70. The flattening of the curves for salt concentrations between 10 and 22.5 per cent salt is due to the fact that a reciprocal reaction took place, *i.e.*, the salt caused an increase in swelling; but because of the higher solution pressure of the salt-acid system this swelling was limited. This same type of reaction is emphasized for the higher concentrations of salt to such an extent that negative swelling results.

From such data, it might appear that it is the amount of acid employed that is the predominating factor as regards swelling. Loeb's[6,24] work on proteins and Küntzel's studies on purified hide substance suggest that the hydrogen-ion concentration is the determing factor for swelling of proteins in acid. The work of Theis and Goetz shows both the initial and equilibrium pH values of the various pickles used. These data are given in Tables 117 to 122, columns c and d, and in Figure 72. From Figure 72 it will be noted that minimum swelling of skin in acid no salt occurred at a pH of 5.3. It can be seen that a pH of 1.5 corresponds to an initial acid concentration of 3.5 per cent, and that the point of minimum swelling (pH 5.3) occurs when the skin contains for all practical purposes little or no acid. It is also quite evident that the pH for minimum swelling decreases with additional concentrations of salt.

Theis and Goetz particularly point out that the swelling of hide or skin is a function of the amount of acid per given weight of the skin and of the pH value of the solution, whereas the prevention of swelling is purely a function of salt concentrations of the pickle solution.

The Sulfuric Acid-Sodium Chloride System

From the practical viewpoint, the sulfuric acid-sodium chloride pickles are of most interest, since they are the ones most used by American tanners.

Theis and Goetz[14] in 1932 made an investigation of sulfuric acid-sodium chloride pickles. For this study, they employed a technique similar to that used in their previous work and dealt with the reaction of pickle liquors with skin and hide. Their data are given in Tables 124 to 130 and in Figures 73 to 80. Theis and Goetz found that the outstanding effect of salt upon

* As a matter of fact, it was Procter's desire to explain the pickling process which led him into the whole field of protein swelling.

Table 124. One Millimol H_2SO_4 per Liter (3.57 Millimols H_2SO_4 per 100 grams dry bated stock).

NaCl Used: Millimols per liter (a)	NaCl Used: Grams per 100 gms bated stock (b)	pH of Pickle: Before (c)	pH of Pickle: After (d)	% gain: Weight (e)	% gain: Vol (f)	M mols H_2SO_4 consumed per 100 gms dry stock (g)	% original H_2SO_4 consumed (h)	Mgs nitrogen per 100 grams dry stock (i)	Grams NaCl adsorbed per 100 grams bated dry stock (j)
0	0	2.71	4.5	2.7	2.6	3.21	90.0	19.9	
75	4.4	2.71	6.58	0	0	3.13	87.6	20.0	0.42
150	8.8	2.70	6.80	0	0	3.16	88.5	40.0	0.73
250	16.1	2.68	6.85	0	0	3.16	88.5	44.8	1.75
350	20.5	2.67	7.0	0.1	0	3.18	89.0	49.8	1.56
500	29.2	2.65	7.0	0	0	3.18	89.0	45.0	2.08
750	44.0	2.64	7.2	1.0	0	3.42	95.6	49.8	3.38
1000	58.5	2.62	7.1	1.5	0	3.40	95.2	60.0	3.50

Table 125. 2.5 Millimols H_2SO_4 per Liter (8.93 Millimols H_2SO_4 per 100 grams dry bated stock).

(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)	(j)
0	0	2.33	3.79	7.3	6.2	8.1	90.7	14.9	
75	4.4	2.33	6.54	2.0	2.6	8.3	93.0	24.0	0.45
150	8.8	2.33	5.00	1.3	0	8.4	94.0	29.8	1.30
250	16.1	2.29	4.62	0.1	0	8.45	94.6	45.0	0.87
350	20.5	2.28	4.90	0	0	8.60	97.3	50.0	2.08
500	29.2	2.27	5.29	0	−2.4	8.50	95.2	—	2.14
750	44.0	2.27	4.95	−1.2	−5.0	8.47	95.0	50	3.25
1000	58.5	2.24	4.81	−0.1	−7.4	8.47	95.0	58	3.76

Table 126. Five Millimols H_2SO_4 per Liter (17.85 Millimols H_2SO_4 per 100 grams dry bated stock).

(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)	(j)
0	0	2.08	3.12	9.5	9.7	15.9	89.2	35.2	
75	4.4	2.04	3.47	4.0	3.6	16.1	90.2	50.0	0.58
150	8.8	2.00	3.77	0.1	2.6	16.9	94.7	62.5	0.82
250	16.1	1.99	3.77	0	0	17.1	95.8	60.0	1.06
350	20.5	1.98	3.98	−0.1	−1.4	17.2	96.4	65.0	2.27
500	29.2	1.96	4.15	−2.5	−2.5	17.3	97.0	54.2	3.48
750	44.0	1.91	4.13	−5.8	−7.4	17.3	97.0	60.0	3.96
1000	58.5	1.84	4.13	−5.0	−6.0	17.3	97.0	56.7	4.8

Table 127. Ten Millimols H_2SO_4 per Liter (35.7 Millimols H_2SO_4 per 100 grams dry bated stock).

0	0	1.84	2.54	11.0	12.0	28.4	79.5	66.6	
75	4.4	1.81	2.70	5.0	6.2	29.5	82.6	66.0	0.67
150	8.8	1.74	2.70	1.3	2.6	30.2	84.6	67.0	1.19
250	16.1	1.70	2.70	1.3	2.6	30.2	84.6	67.5	2.40
350	20.5	1.67	2.74	2.6	2.6	30.4	85.2	69.2	3.34
500	29.2	1.64	2.65	−1.5	−2.5	30.5	85.5	65.0	3.53
750	44.0	1.61	2.72	−4.0	−5.0	31.6	88.5	65.0	4.55
1000	58.5	1.58	2.77	−4.3	−7.4	32.1	90.0	65.0	7.00

Table 128. Twenty Millimols H_2SO_4 per liter (71.4 Millimols H_2SO_4 per 100 grams dry bated stock).

0	0	1.56	2.06	13.3	15.7	48.5	67.9	113.2	
75	4.4	1.53	2.04	11.5	9.7	49.8	69.7	118.0	0.57
150	8.8	1.51	2.04	6.4	7.3	48.8	68.3	119.0	0.85
250	16.1	1.49	2.03	5.5	7.3	48.6	68.0	108.5	0.98
350	20.5	1.43	2.03	4.5	2.5	49.0	68.6	123.5	1.30
500	29.2	1.41	2.01	0.1	−2.5	49.0	68.6	124.3	1.36
750	44.0	1.37	1.91	−3.3	−6.3	48.5	67.9	125.2	2.34
1000	58.5	1.32	1.84	−1.0	−5.0	48.5	67.9	120.0	4.15

Table 129. Thirty Millimols H_2SO_4 per Liter (107.0 Millimols H_2SO_4 per 100 grams dry bated stock).

0	0	1.40	1.70	12.2	15.7	54.5	51.0	127.2	
75	4.4	1.39	1.70	9.0	12.2	52.0	48.6	128.2	0.35
150	8.8	1.37	1.69	8.5	7.3	51.7	48.3	134.2	0.97
250	16.1	1.34	1.63	6.2	7.3	51.5	48.2	134.2	1.17
350	20.5	1.32	1.61	2.7	2.5	52.5	49.2	128.2	1.30
500	29.2	1.29	1.59	−0.9	−2.5	51.4	48.1	140.0	1.30
750	44.0	1.22	1.58	−4.3	−7.7	51.3	48.0	139.5	2.47
1000	58.5	1.17	1.54	−1.9	−5.0	52.3	48.8	135.5	4.83

Table 130. Forty Millimols H_2SO_4 per Liter (142.8 Millimols H_2SO_4 per 100 grams dry bated stock).

NaCl Used		pH of Pickle		% gain		M. mols H_2SO_4 consumed per 100 gms dry stock	% original H_2SO_4 consumed	Mgs nitrogen per 100 grams dry stock	Grams NaCl adsorbed per 100 grams bated dry stock
Millimols per liter (a)	Grams per 100 gms bated stock (b)	Before (c)	After (d)	Weight (e)	Vol (f)	(g)	(h)	(i)	(j)
0	0	1.28	1.51	11.2	12.2	54.0	37.9	108.5	
75	4.4	1.26	1.47	7.6	9.7	52.0	36.5	119.0	0.52
150	8.8	1.24	1.43	8.6	7.3	53.5	37.5	119.0	0.91
250	16.1	1.22	1.41	7.6	7.3	53.2	37.2	108.5	1.88
350	20.5	1.20	1.39	6.2	5.0	53.2	37.2	104.6	2.21
500	29.2	1.16	1.37	−0.8	−2.5	52.8	37.0	100.0	2.47
700	44.	1.10	1.32	−3.8	−10.0	52.4	36.7	100.0	2.73
1000	58.5	1.09	1.29	−2.5	−5.0	52.4	36.7	100.0	5.33

hydrochloric acid absorption was that the amount of acid absorbed due to salt was a function of the sodium chloride concentration. Furthermore, the amount of acid absorbed due to salt increased to a maximum and then decreased. This phenomenon was partially explained on the basis of the Donnan Membrane Equilibria Theory, in which the increase in acid absorbed due to salt functions to maintain a chloride equilibrium between the internal

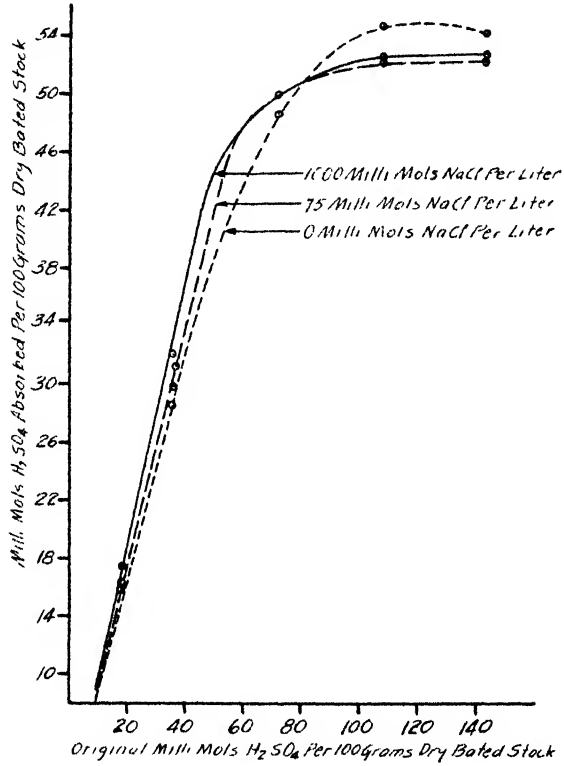


Figure 73

and external phases. On this basis, one might expect that a similar result would obtain for the sulfuric acid-sodium chloride pickles. Theis and Goetz found that this is not the case. Reference to the data given in Tables 124 to 130 and in Figures 73 and 74 shows that salt has but very little effect upon increasing acid absorption. Kuntzel and Priesentanz working with collagen noted that sodium chloride had no effect on sulfuric acid absorption.

In considering and interpreting the reaction of the two systems, H_2SO_4—NaCl and HCl—NaCl, the following may be set down in rather categorical fashion:

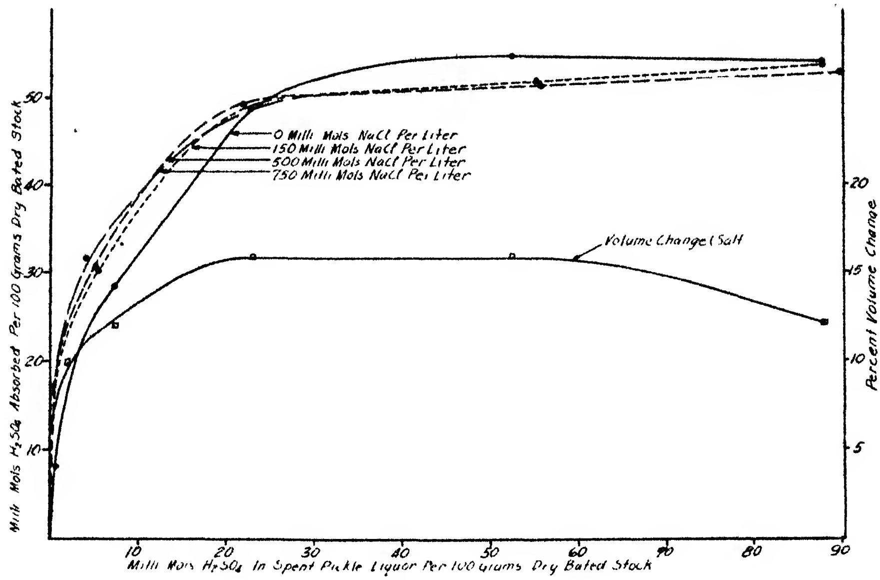


Figure 74

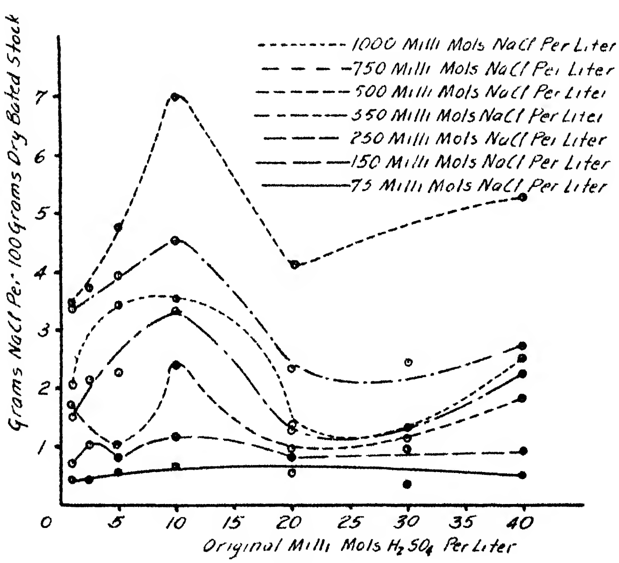


Figure 75

(1) There is not the common ion effect in the system H_2SO_4 -NaCl as there is in the HCl--NaCl one. In the former, the divalent $SO_4^{=}$ ion tends to increase the H^+ ion absorption to a greater degree than the monovalent Cl^- ion. Consequently, any effect that salt (NaCl) may have on increasing acid absorption to a maximum should occur at a sulfuric acid concentration much less than that concentration of hydrochloric acid necessary for maximum absorption.

(2) In most adsorption phenomena involving acids, the H^+ ion of the

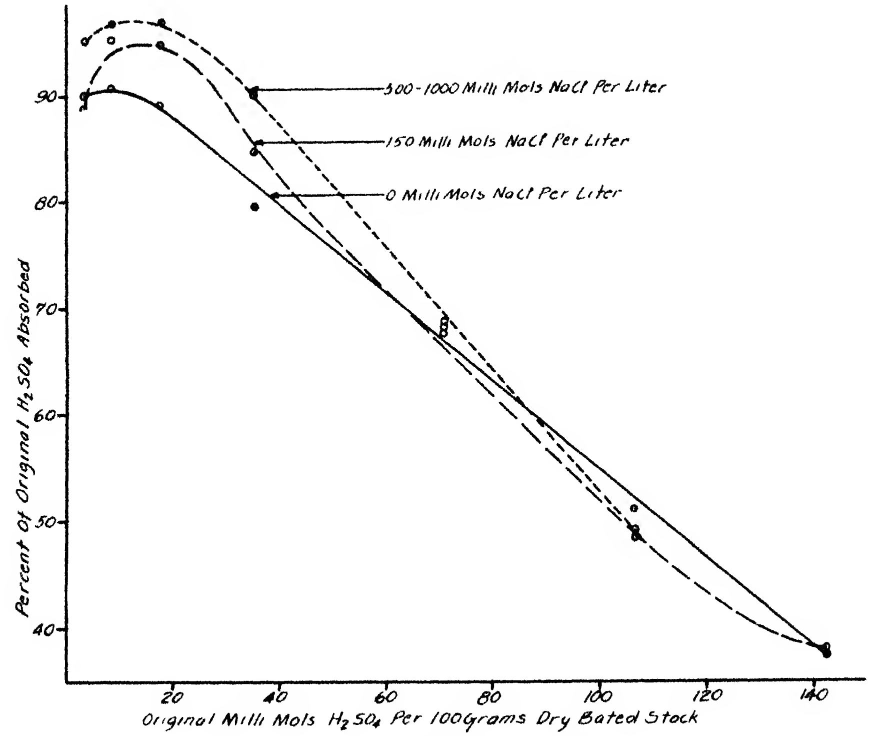


Figure 76

acid is adsorbed to a greater degree in the presence of anions different from the anion of that acid. Due to the presence of sodium chloride in the H_2SO_4—NaCl system, such a condition would be induced.

(3) With increasing valency there is an increase in adsorption equivalents, particularly at low concentrations of acid. Hoffmann and Gortner,[4] working with gelatin, showed that for high concentrations of electrolytes typical adsorption takes place. Küntzel and his collaborators maintain that the Donnan Equilibria Theory does not apply to acid-salt-collagen systems.

In all probability the high concentrations employed in their work and the adsorptive phenomena have a considerable effect in this respect. The Donnan effect has been applied by workers in protein chemistry to the colloidal state of protein dissolved in water, whereas Martin Fischer[2A] maintains that in dealing with skin collagen, the condition is similar to a water-in-protein system, resulting in a concept that should tend toward the application of the laws of concentrated solutions rather than those of dilute solutions. Theis and Goetz, however, maintain that the Donnan concept should obtain.

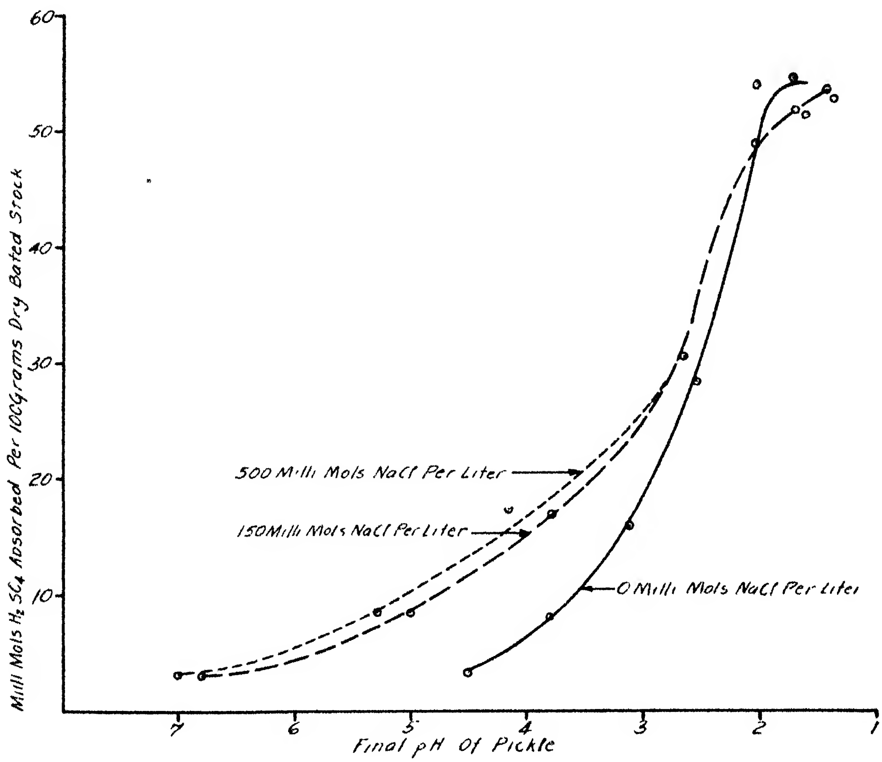


Figure 77

Theis and Goetz show in Figure 74 that there is a slight increase in sulfuric acid absorbed due to salt at low concentrations of acid and that, furthermore, this increase is greatest for the original acid concentration of 10 millimols per liter. Not only was the acid absorption greatest at this point but likewise the salt absorption, as can readily be seen from Figure 75. The general trend of the salt curves and the acid curves correlated with protein sol formation tend to indicate that absorption of both acid and salt are

dependent, at least to some degree, upon the number of protein linkages available, which are controlled by the acid and salt concentration. This postulation is in agreement with the expressed ideas of Thomas[22] and Gortner[3] in that apparently the Cl^- ion causes an increase in protein hydrolysis, whereas the effect of the $SO_4^{=}$ ion is almost negligible. The presence of both Cl^- and $SO_4^{=}$ ions in a sulfuric acid pickle causes an "antagonistic" effect.

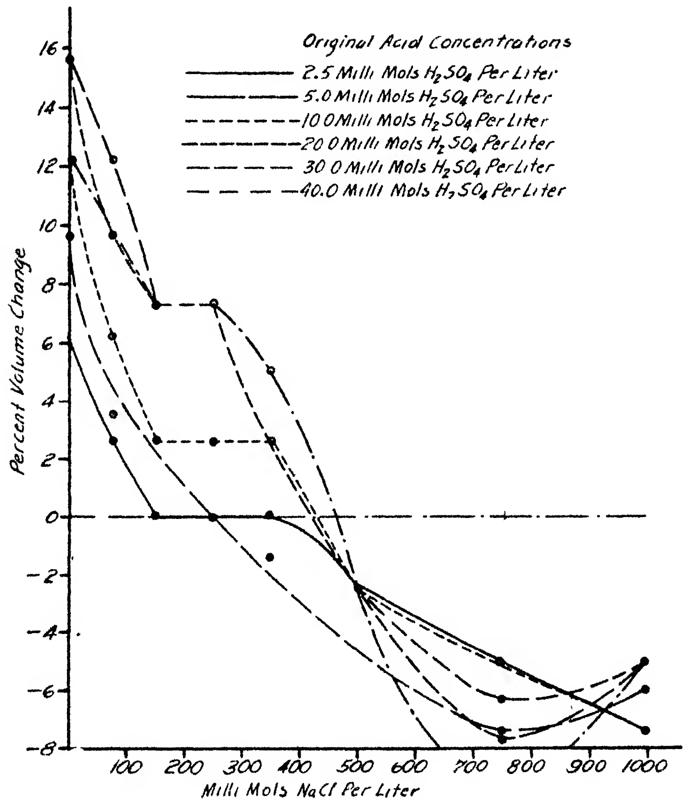


Figure 78

Theis and Goetz found that maximum acid absorption was obtained with with an original acid concentration of 20 millimols H_2SO_4 per liter or with 71.4 millimols H_2SO_4 per 100 grams of dry bated skin. Any amount of acid in excess of that required for maximum absorption was unnecessary insofar as complete pickling was concerned. The per cent of original acid consumed by pickling with the various acid and salt combinations is shown in Figure 76.

Skins that are pickled for preservation after bating would of necessity need to retain a considerable amount of acid. Theis and Goetz noted this

fact in their investigations; hide which had been pickled with 35.7 millimols of acid per 100 grams of dry bated stock became putrid after 4 days' exposure to moist air. In this regard, the salt retained by the skin will be a necessary factor in retarding putrefaction and mold growth.

Due to the slight influence upon sulfuric acid absorption, the effect of salt upon the pH values of the equilibrium pickle was more pronounced than the change of pH value that would result from acid absorption due to the amount

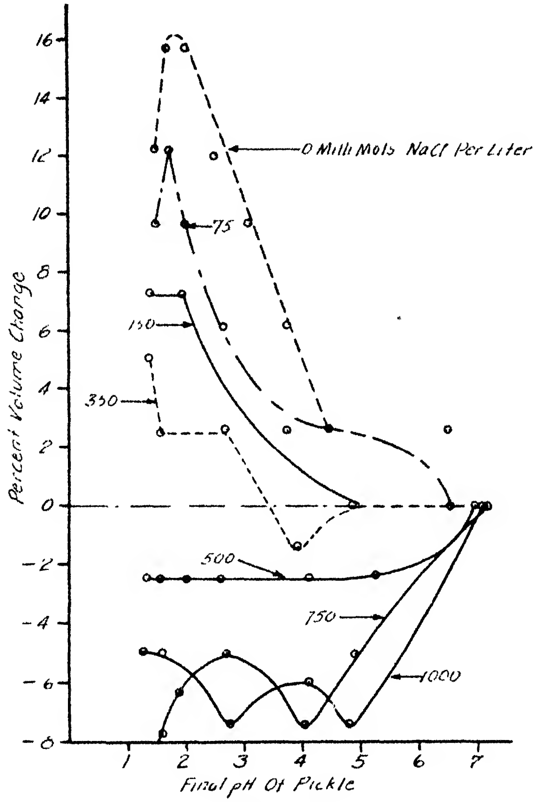


Figure 79

of salt in the pickle, as was the case with hydrochloric acid. This difference can be observed by a study of the pH values of the pickle solutions before and after pickling as recorded in columns c and d of Tables 124 to 130. The difference in pH values for the different salt concentrations may be attributed chiefly to the effect of salt upon the increase in H^+ ion activity, because the amount of acid absorbed was practically the same for the different salt concentrations. It should be emphasized that this effect was more pronounced

for the higher concentrations of acid, and is a factor that should be considered in the control of sulfuric acid-sodium chloride pickle solutions, particularly when the amount of skin placed in such pickle liquors varies and when, in consequence, the ratio of skin to volume of liquor becomes a variable quantity. Figure 77 shows that maximum acid absorption occurs between the values of pH 2.0 and pH 1.5, and even though this is an apparently small range, it required originally the addition of twice as much acid to give the lower pH value.

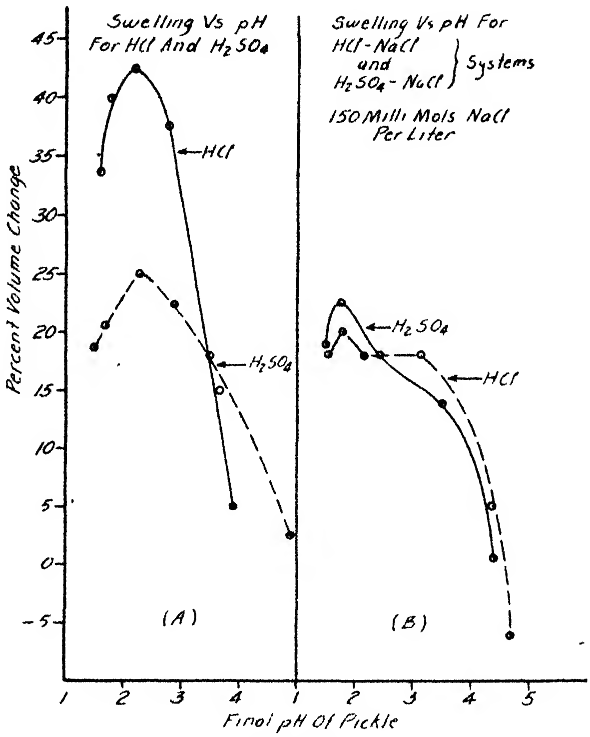


Figure 80

The swelling, in terms of weight gain and volume change, taking place in the various sulfuric acid-salt pickles, is shown in columns e and f of Tables 124 to 130 and in Figures 78 to 80. From these data, it can be observed that for the highest concentration of acid used, namely, 40 millimols H_2SO_4 per liter, or 122.8 millimols per 100 grams dry bated skin, about 475 millimols of salt per liter was sufficient to prevent swelling. In general, 400 to 500 millimols of salt were sufficient to prevent swelling for all concentrations of acid used. High concentrations of soldium chloride resulted in a dehydrating effect. Comparing such data with that obtained for hydrochloric acid, it is

seen that the same concentrations of salt were sufficient to prevent swelling for all the concentrations of acid employed.

Figure 79 shows the effect of salt concentration upon swelling when the data were plotted versus equilibrium pH value. Again, the similarity of the trend of these curves to those of hydrochloric acid is apparent. However, there is this significant difference: the maximum swelling of skin in a sulfuric acid solution (no salt) is considerably less than that with hydrochloric acid. Figure 80 shows this comparison. It can readily be seen from part A of this figure that there is divergence from the osmotic effect, which is particularly evident at pH values greater than 3.3, where the volume change with hydrochloric acid was less than with sulfuric acid. The specific ion effect upon protein peptization and upon adsorptive phenomena no doubt plays an important role in this capacity also. It is interesting to note that even though the hide showed different swelling capacities in the different acids, the depression value of swelling by salt was practically the same. This would be expected from a consideration of both the osmotic concept and the alternative theory of swelling worked out by Tolman and Stern,[23] and based only upon adsorptive phenomena.

The Time and Temperature Effect

Theis and Goetz[15] in 1932 made a study of the time and temperature effect upon the pickling of bated skin. Their technique was as follows: 25-gram samples of bated skin were placed in 250 ml of pickle solution, containing 25 millimols of sulfuric acid per liter (83.2 millimols acid per 100 grams dry bated skin) and 500 millimols of sodium chloride per liter. The initial pH value of the pickle was 1.43. A series of such samples was then placed in thermostats at various temperatures and allowed to remain for varying periods of time. The pickled skin was removed from the pickle solution and the residual liquor analyzed for acid, salt, dissolved nitrogen and equilibrium pH value. The skin samples were surface-dried, and the percentage volume and weight gain were determined. The data obtained are shown in Table 131 and in Figures 81 and 82.

If Table 131 is analyzed, it is seen that both temperature and time play a part in pickling. With regard to acid absorbed over a temperature range, it may be readily seen that in the early stages of pickling, temperature increases the rate of acid absorption. Beyond 8 hours' contact with the pickle solution, acid absorption becomes more or less constant for all the temperatures used. For any given time interval, such as 1, 2, 4 or 8 hours, temperature increase up to 32° apparently increases acid absorption; beyond 32°, increase in temperature causes decreased acid absorption and marked increase in dissolved nitrogen. These data are shown graphically in Figure 81. Column g (Table 131) shows the salt absorption and indicates that most

Table 131. Effect of Time and Temperature on Pickling of Steer Hide

Original Pickle Solution:
500 Millimols NaCl per Liter
25 Millimols H_2SO_4 per Liter (83.2 Millimols per 100 gms dry bated hide)
pH—1.43)

Hrs in pickle (a)	Temperature of pickle (° C) (b)	—Per cent Change— Wt (c)	Vol (d)	Final pH of pickle (e)	Millimols H_2SO_4 consumed per 100 gms dry stock (f)	Grams NaCl consumed per 100 gms stock (g)	Mg nitrogen per 100 gm dry stock (h)
	7.0	8.8	6.4	1.63	29.7	0.94	14.0
	20.0	6.4	4.3	1.67	31.6	1.17	14.0
1	25.0	6.8	4.3	1.69	32.9	1.09	23.2
	32.2	7.6	4.3	1.77	36.2	1.02	32.6
	37.5	3.6	4.3	1.63	34.8	1.17	111.0
	7.0	8.0	5.0	1.69	36.2	1.02	18.6
	20.0	6.4	4 3	1.72	40.8	1.25	32.6
2	25.0	6.0	3.7	1.79	42.8	1.09	32.6
	32.2	6.2	2.8	1 77	43.5	1.25	46.5
	37.5	2.0	0.0	1.82	42.8	1.17	216.0
	7.0	7.6	4.3	1.76	44.8	1.25	32.6
	20.0	6.4	4 3	1.79	46.7	1.17	32.6
4	25.0	5.6	0.0	1.81	48.0	1.33	32.6
	32.2	6.0	0 0	1.84	49.5	1.25	46.5
	37.5	0.4	−4.3	1.84	46.5	1.33	381.0
	7.0	6.4	2.2	1.86	50 6	1.48	32.6
	20 0	6.4	2.2	1.89	50.6	1.02	41.8
8	25.0	2.4	−2 2	1 84	50.6	1.40	46.5
	32.2	4.4	−2.2	1.84	51.5	1.29	81.5
	37.5	1.3	−4.3	1.84	—	1.21	768 0
	7.0	6.4	2.2	1.87	52.1	1 25	37.1
	20.0	4.3	0.0	1.82	51.5	1 09	37.1
12	25 0	5 1	0.0	1.91	53.4	1.17	46.5
	32 2	6.4	4.2	1.87	52.1	1.33	112.0
	37.5	−2.8	−8.8	1.87	48.0	1.17	1090.0
	7.0	4.4	2.2	1.87	52.7	1.25	46.5
	20.0	3.7	2.2	1.91	50.8	1.02	46.5
18	25.0	2.8	0.0	1.87	51.5	1.17	46.5
	32.2	4.4	0.0	1 91	52 0	1.17	144.0
	37.5	−6 5	−8.8	1.81	44.8	1.17	
	7.0	3.1	0.0	1.79	53.2	1.17	51.2
	20.0	4.8	0 0	1.76	48 6	1.29	53.5
25	25 0	2.3	−2.2	1.82	54.7	1.29	81.4
	32.2	5.5	0.0	1.79	51.6	1.17	241.0
	37.5	−8.0	−10.5	1.84	48.3	1.40	2310.0

of the salt is taken up by the skin immediately after immersion in the pickle liquor. It would thus appear that neither time nor temperature plays much of a part in this case.

The effects of time and temperature are both apparent in the case of weight and volume change of the skin. Temperatures below 10° apparently increase the weight and volume change; but above this temperature, little or no change is noted until the temperature is increased beyond 32°. In all cases at 37.5°,

the weight and volume change was drastically decreased. This is to be noted from columns c and d of Table 131 and also in Figure 82. If we follow this weight and volume change, for any given temperature, over a period of pickling time, we find that hydration and swelling occur within the first hour of pickling, beyond which time the swelling steadily decreases. Such initial

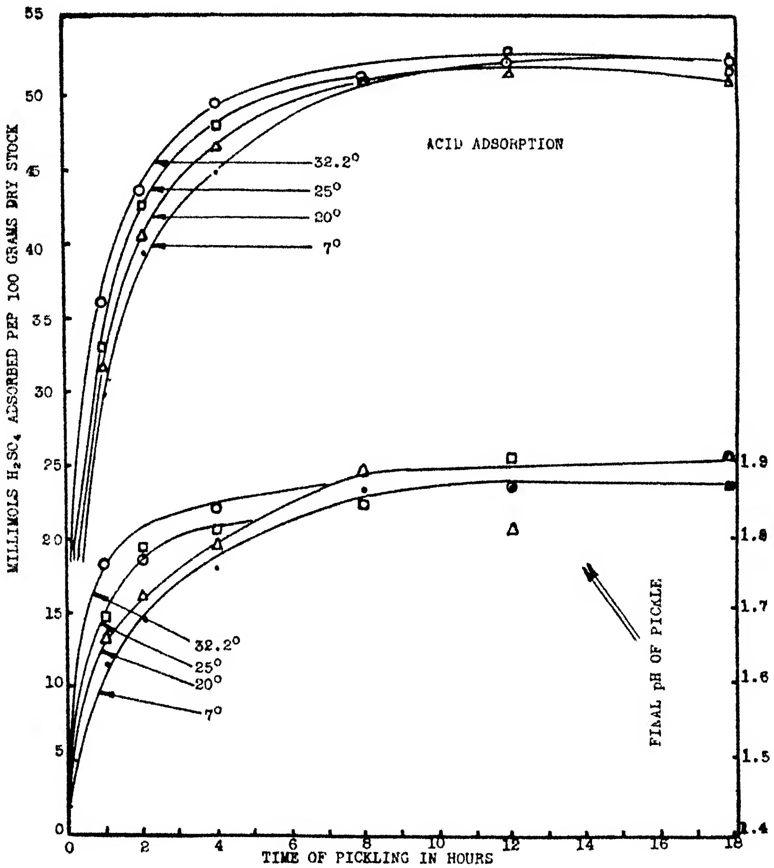


Figure 81. Effect of Time of Pickling upon Acid Adsorption and upon Final pH of Pickle for Various Temperatures.

swelling (during the first hour) is undoubtedly due to the very rapid absorption and combination of the acid by the skin which occurs during the early part of the pickling period. Beyond this period the dehydration effect of the salt comes into play, and a shrinkage of the skin takes place.

Column e of Table 131 shows the change in final pH value of the pickle liquor with time and temperature. It is obvious from these data that the

final pH value of the pickle solution is increased with both time and temperature during the early stages of pickling. This effect of temperature is not

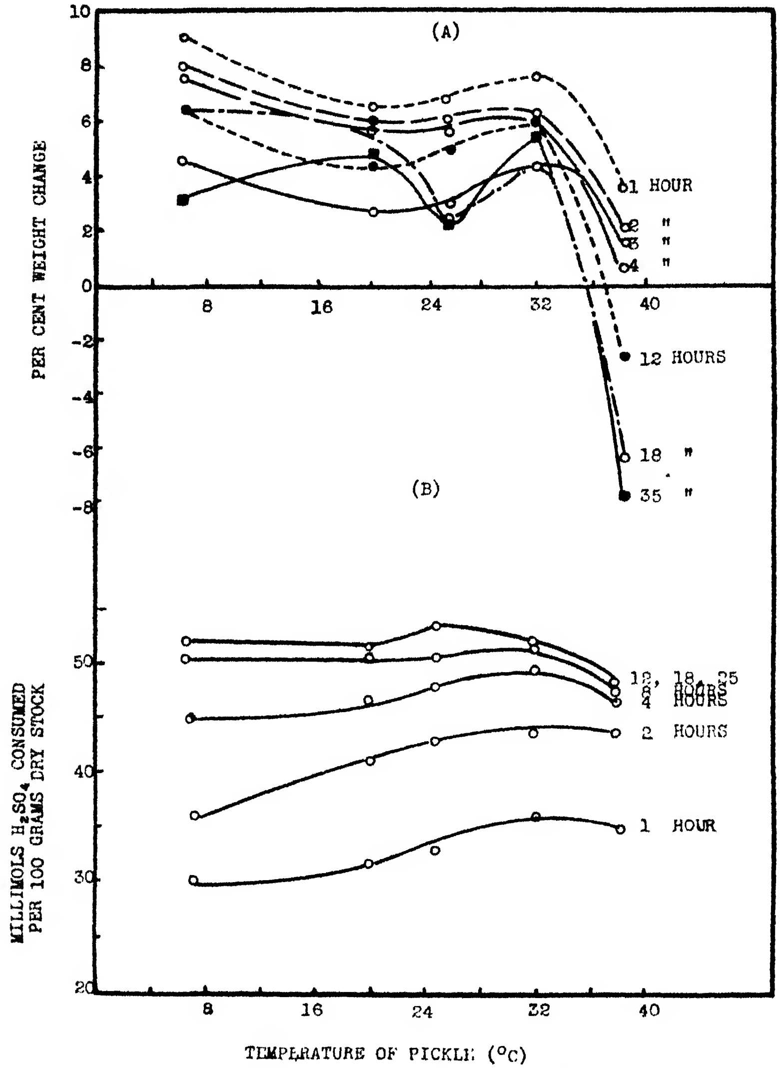


Figure 82. Effect of Temperature of Pickle upon Weight Change and upon Acid Adsorption after Various Periods of Time of Pickling.

apparent however, in the case of 18 and 25 hours of pickling, probably because an equilibrium state was attained during this increased time, regardless of temperature of reaction.

Hydrolysis and Peptization of Skin

If residual pickle liquor is allowed to stand for a length of time or is heated, coagulation of the soluble proteins usually occurs. This coagulated substance undoubtedly represents: (a) coagulable proteins remaining within the skin after bating and dissolved from the skin by the salt solution during pickling; (b) simplified proteins, produced by acid hydrolysis and thus rendered soluble in the pickle liquor; and (c) coagulated proteins, existing within the skin, which are resistant to the action of the soaking process, but are partly reversed to the non-coagulated state by the action of the alkaline lime and bate, and then dissolved out by the salt during pickling.

Theis and Goetz[14] gave some little attention to the combined effect of salt and acid upon protein sol formation and the resulting action between the acid-salt system and hide substance. Previous workers have more or less neglected this phase of pickling. Thomas and Foster[22] found that all halides increase the hydrolysis of hide substance, whereas sulfates inhibit hydrolysis in neutral solutions. Gortner, Hoffmann and Sinclair,[3] in an extensive study on the effect of different salts and different salt concentrations upon sol formation, found that the degree of peptization was due to the specific influence of the anions which were present in equivalent concentrations. Pfeiffer, Wurgler and Witka[8] found that definite and fairly stable compounds were formed between halogen salts and amino acids, with a considerable increase in solubility of the amino acids. The work of Osborne[8] on edestin showed that the change in globulin by salt was affected by the kind of salt and the amount of acid, and that the H^+ ion might be the active catalyst. Changes in hydrogen ion concentration may bring about molecular rearrangement, such as a shift in the keto $\rightleftharpoons$ enol isomerism, whereby additional reactive groups are formed within the protein molecule. The cause of the variations obtained with different salts and acids upon protein rearrangement and peptization is in all probability very complex, involving the structural cohesional forces within the protein network, secondary valence, polar groups, degree of hydration, specific ion effect, electrokinetic forces, adsorptive effects, and others still unknown. Based upon the work of Stiasny, Pfeiffer and others, Gustavson[8] reasoned that the first effect of a neutral salt upon protein rearrangement is the combination of the salt with the polypeptide chain; this lessens the attraction of these units for one another by using up some of the secondary valence forces which initially bound them together.

The foregoing definitely indicates that the pickling process is essentially a very complex one; and it might be reasoned that the action of salt and acid is to cause a break-down of the skin collagen in such a way as to bring about combination of the acid and salt at certain centers which were previously utilized as cohesional bonds between the polypeptide chains. In the pH range 1.5 to 2.5, normal pickling does not cause additional acid to bind with

the protein over that normally obtaining for that particular pH value, in the absence of added salt. The effect of salt is twofold: the one to control

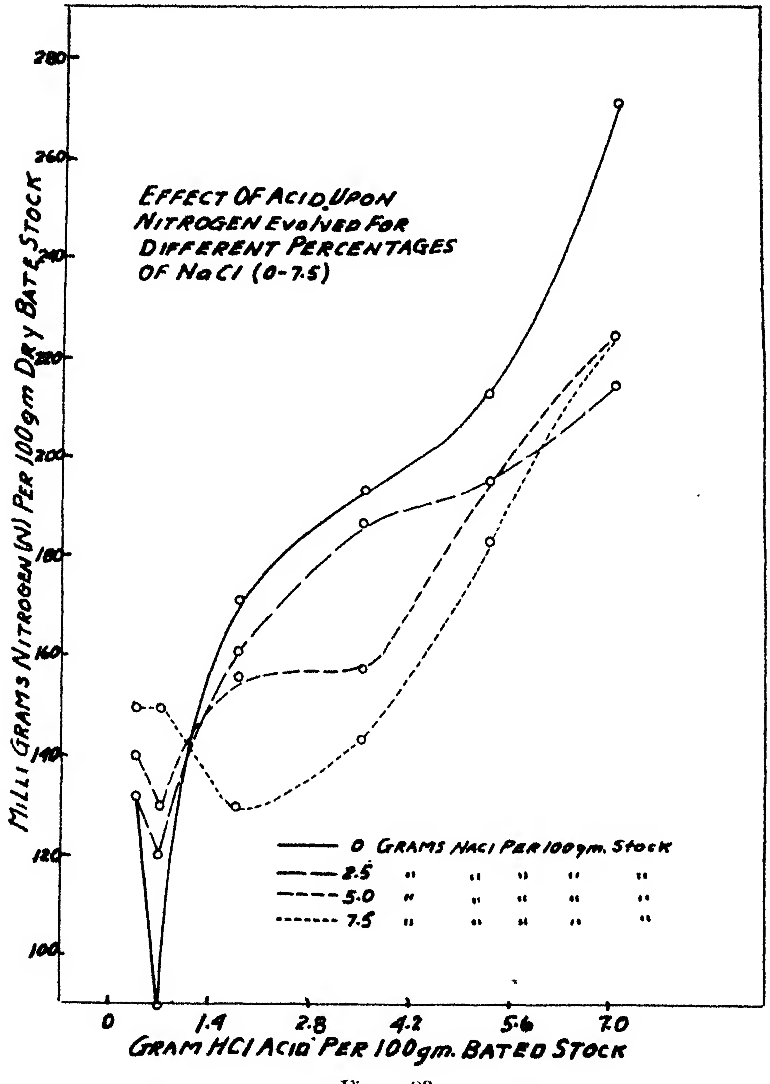


Figure 83

swelling, and the other to cause even distribution in acid-fixation throughout the skin.

Theis and Goetz determined the dissolved nitrogenous substances in both their HCl–NaCl and H_2SO_4–NaCl pickle solutions after equilibrium had

been attained. Figures 83 and 84 show such data as were obtained. For the HCl—NaCl systems, these investigators note the following two main facts. The first is that, at very low acid concentrations, the addition of salt greatly increases the dissolved nitrogen. This is evident for all salt concentrations, the dissolved nitrogen reaching a maximum value at about 15 per cent salt. The second fact is that, at higher acid concentrations, the dissolved nitrogen for

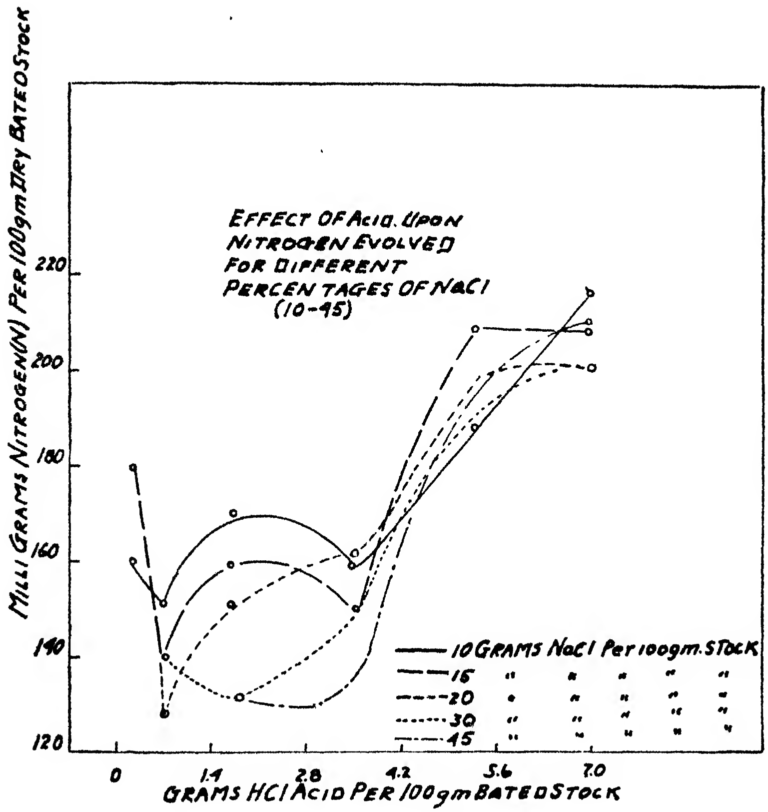


Figure 84

any salt concentration decreases below that of 0 per cent salt, undoubtedly because of the retardation of the hydrolysis effect of the acid present. The dissolved nitrogen, however, for any given salt concentration increases with the increase in acid content, each increase in salt content decreasing the amount of dissolved nitrogen below that of the next lowest salt concentration.

Thus, with the HCl—NaCl pickle solutions, the effect of salt was, in general, to decrease formation of protein sol, as gauged by dissolved nitrogen

content. However, in the range of maximum acid-absorption due to salt concentration, the sol formation was more or less constant.

Figures 85 and 86 indicate the hydrolysis taking place in the sulfuric acid-salt pickles. It can be seen that there is a slight increase in protein sol formation with increase in salt, and a decided increase with increase in acid up to 30 millimols per liter, and then a decrease. In Figure 66 it was shown

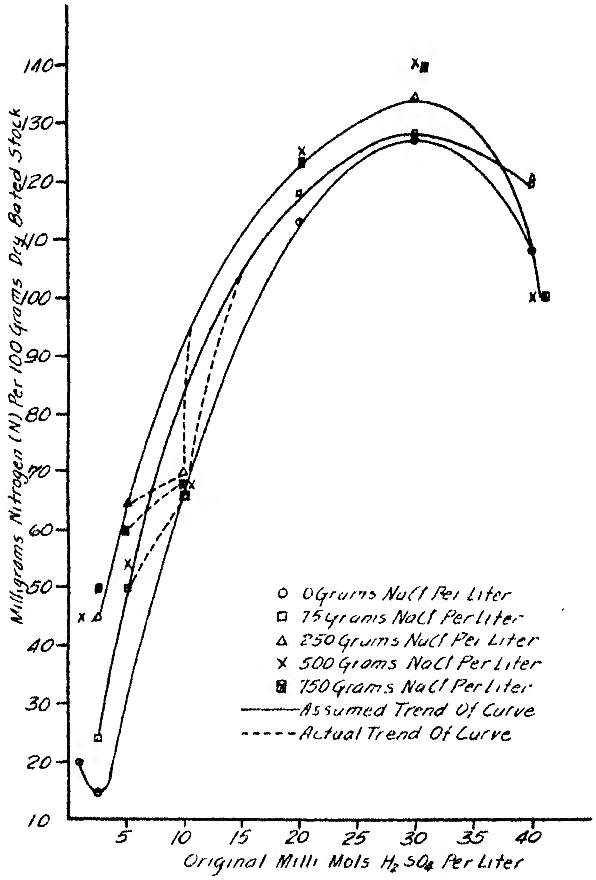


Figure 85

that there is a slight increase in acid absorbed, due to salt at low concentrations of acid, and that this increase is greatest for an initial acid concentration of 10 millimols per liter. At this concentration of acid, the effect of salt upon protein hydrolysis appears to be negligible. However, at concentrations greater than 10 millimols acid per liter, sodium chloride has some small effect upon the amount of nitrogen dissolved from the skin, but it would appear

that the initial hydrochloric acid concentration plays the more predominant role in sol formation.

Since the acid and salt content of the pickle play a role in the peptization of the proteins, this factor must be affected by time and by temperature. Theis and Goetz studied this factor, and the data are shown graphically in Figure 87. These data show that in the early stages of pickling, and at any

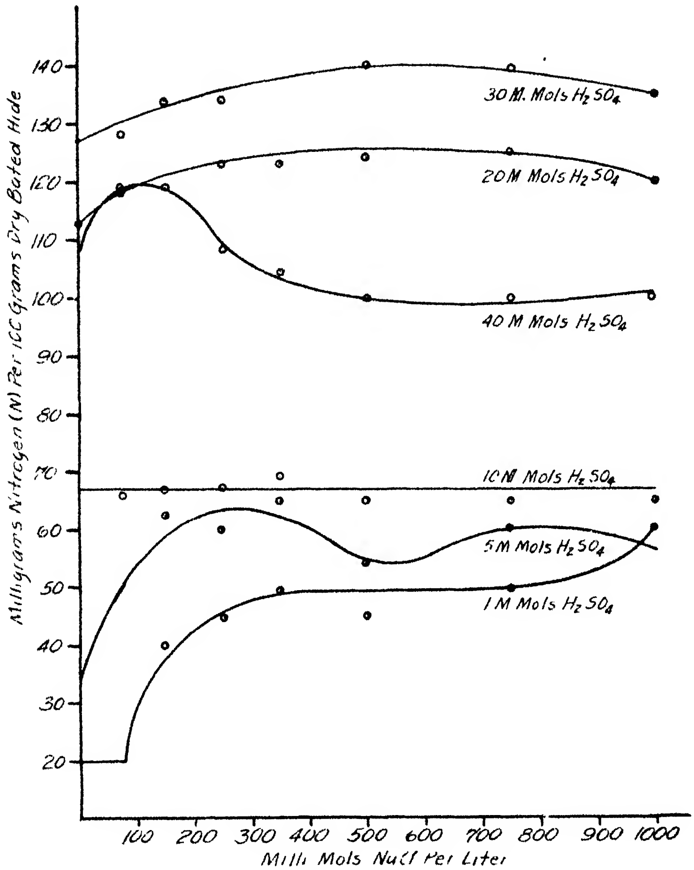


Figure 86

reasonable temperature, the amount of dissolved nitrogen increases almost as a linear function with a steep slope; but that after some 4 hours, the slope decreases and the curves flatten and appear to assume an asymptotic condition. This is particularly the case of temperatures of 8°, 20°, and 25°. At 32 and 37°, the hydrolysis and peptization of the proteins is greatly increased and is, in all probability, due to the actual break-down of the collagen of the skin, such as would be expected in an acid solution at such temperatures.

This temperature factor is an important one, since in the summer season some tanners use water which is exposed to the atmosphere for a long time, and may thus easily have a temperature greater than 25°.

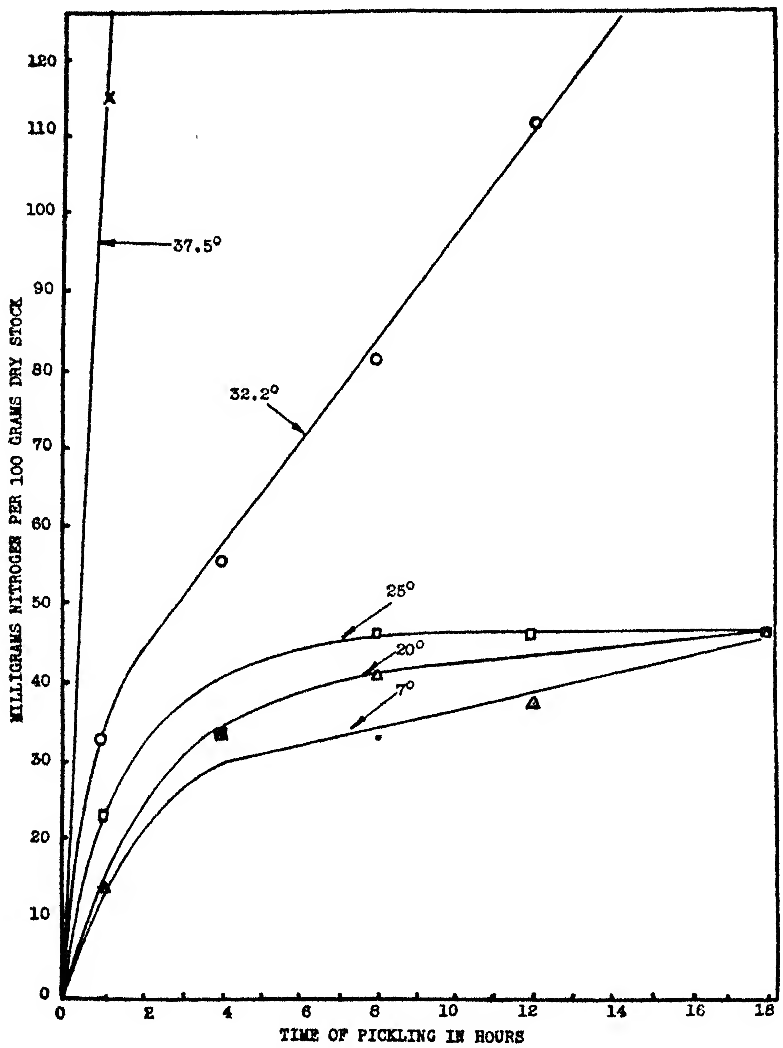


Figure 87. Effect of Time of Pickling upon Nitrogen Sol Formation for Various Temperatures of Pickle.

In general, and by a comparison of protein hydrolyzed by the HCl—NaCl pickle, the H_2SO_4—NaCl system should give a more solid and firmer piece of leather, and this condition is manifested in plant practice. Practice has

Table 132. 0.5% HCl on the Weight of Bated Stock (69% Moisture) 30% NaCl and 44.2 Millimols HCl on 100 Grams Dry Stock.

Ratio of liquor to skin	pH of solution before	pH of solution after	% Gain in weight	% Gain in volume	HCl Initial concentration millimols per liter	HCl Millimols consumed for 100 gms dry stock	HCl % consumed	NaCl Initial concentration millimols per liter	NaCl % consumed	Gms NaCl consumed 100 gms bated stock
2: 1	1.03	3.82	− 0.666	− 4.76	68.5	43.14	97.7	2564.0	26.50	7.95
4: 1	1.52	3.83	− 5.11	− 5 95	34 25	42.86	97.2	1282.0	17.50	5.25
6: 1	1.76	3.84	− 8.45	− 8.33	22.80	42 36	96.2	854.5	14.32	4.30
10: 1	2 00	3.83	− 8.90	− 9.52	13.70	41 34	93.7	513.0	10 68	3.20
15: 1	2.20	3.77	− 7.77	− 7.14	9.13	41.80	95.0	342.0	10.00	3.00
20: 1	2 29	3 69	− 7.12	− 7.14	6.85	40.90	92 7	256.0	8.33	2 50

Table 133. 1.0% HCl on Bated Weight (69% Moisture) 30% NaCl on Bated Weight 88 2 Millimols HCl on 100 Grams Dry Stock.

Ratio of liquor to skin	pH of solution before	pH of solution after	% Gain in weight	% Gain in volume	HCl Initial concentration millimols per liter	HCl Millimols consumed for 100 gms dry stock	HCl % consumed	NaCl Initial concentration millimols per liter	NaCl % consumed	Gms NaCl consumed 100 gms bated stock
2: 1	0.73	2.88	+ 1.55	− 4.76	137	86.69	98.2	2564.0	27.8	8.35
4: 1	1.23	2.90	+ 0.666	− 0.00	68.5	86.30	97.6	1282.0	19.7	5.90
6: 1	1.48	2.98	+ 0.0	− 3.57	45.60	85.47	96.6	854.5	14 5	4.35
10: 1	1.73	2.90	− 4.00	− 2.38	27 40	82 90	93.8	513.0	9.17	2.75
15: 1	1 90	2.85	+ 6.66	+ 5.95	18.60	80.62	91.4	342.0	9.34	2.80
20: 1	2 20	2.90	+17.8	+21.4	13.70	77.85	88.1	256.0	6.67	2.00

Table 134. 1.5% HCl on Bated Weight (69% Moisture) 30% NaCl on Bated Stock 132.6 Millimols HCl on 100 Grams Dry Stock).

Ratio of liquor to skin	pH of solution before	pH of solution after	% Gain in weight	% Gain in volume	HCl Initial concentration millimols per liter	HCl Millimols consumed for 100 gms dry stock	HCl % consumed	NaCl Initial concentration millimols per liter	NaCl % consumed	Gms NaCl consumed 100 gms bated stock
2: 1	0.59	1.53	+ 1.56	− 3.57	205.50	121.85	91.8	2564.0	29.8	8.95
4: 1	1.20	1.89	− 1.78	− 4.76	102.75	120.20	91.5	1282.0	14.0	4.20
6: 1	1 30	1.82	− 3.34	− 4.76	68.50	108.20	88.5	854.5	13.3	4.00
10: 1	1.58	2.20	+ 2.22	+ 2 38	41.10	112.20	84.6	513.0	12.3	3.70
15: 1	1.76	2 30	+20.9	+21.4	27.40	109 20	82.5	342.0	12.4	3.72
20: 1	1.84	2 38	+29.3	+33.4	20.55	104.10	78.6	256.0	7.67	2.30

Table 135. 1.0% HCl Based on Bated Weight of Skin (70.0% H_2O) 6% NaCl Solution 88.2 Millimols HCl Based on 100 Grams Dry Bated Skin.

Ratio of liquor to skin	pH value solution final	% gain weight	% gain volume	HCl concentration: Initial millimols per liter	HCl concentration: Millimols absorbed by 100 g dry skin	HCl concentration: Per cent absorbed	NaCl concentration: Initial millimols per liter	NaCl concentration: Millimols actually present	NaCl concentration: Gms NaCl absorbed 100 g bated skin	NaCl concentration: Millimols absorbed 100 g bated skin	NaCl concentration: Per cent absorbed
Bated Skin Not Centrifuged											
2: 1	3.39	− 9.8	− 9.7	137.0	89.0	97.5	1020	204	4.17	71.4	35.0
4: 1	3.41	− 3.0	0.0	68.5	88 2	96.5	1020	408	4.76	81.4	20.0
6: 1	3.07	− 1.0	+ 7.0	45.7	87.4	95.6	1020	612	5.26	90.0	14.7
10: 1	2.99	− 4.2	+ 5.9	27.4	85 9	94.0	1020	1020	5.76	98.2	9.6
15: 1	3.10	+ 2.2	+13.3	18.4	85.3	93.3	1020	1530	6.84	117 2	7.7
20: 1	2.86	+ 0 6	+12.2	13.7	82.8	90.7	1020	2040	7.70	132.2	6 5
Bated Skin Centrifuged (50.0% H_2O)											
2: 1	4.80	−11 6	−13.5	137.0	53.4	97.7	1020	204	5.20	177.8	43.3
4: 1	4.45	−10.4	− 4 6	68.5	53.4	97.7	1020	408	6.30	215.5	26.3
6: 1	4.54	− 7.6	− 2.3	45 7	52.6	96.1	1020	612	6.68	228.5	18.6
10: 1	4.76	0.0	− 1.1	27.4	52.6	96 1	1020	1020	7.76	265.5	13.0
15: 1	5.18	+11.8	+ 9.7	18.4	51.2	93.3	1020	1530	9.98	341.0	11.1
20: 1	3.96	+ 5.6	+ 6.6	13.7	48.6	88.6	1020	2040	10.98	375.0	9.2

Table 136. Comparing Values of Concentration of Acid Inside and Outside of Pickled Skin.

Dilution	C_1	C_2	C_2/C_1	$\log_{10} C_2/C_1$	Volume change
		½ Per Cent Acid			
2:1	34.64	0.96	.0276	−1.5575	−4.76
4:1	34.36	1.24	.0362	−1.4413	−5.95
6:1	33.86	1.74	.0514	−1.2890	−8.33
10:1	32.84	2.76	.0840	−1.0757	−9.52
15:1	33.30	2.30	.0690	−1.1510	−7.14
20:1	32.40	3.20	.0989	−1.0048	−7.14
		1 Per Cent Acid			
2:1	78.20	1.71	.0219	−1.6596	−4.76
4:1	77.80	2.10	.0270	−1.5686	0.0
6:1	76.00	2.93	.0386	−1.4134	−3.57
10:1	74.40	5.50	.0740	−1.1308	−2.38
15:1	72.10	7.78	.1078	−0.9674	+5.95
20:1	69.40	10.55	.1522	−0.8176	+21.4
		1½ Per Cent Acid			
2:1	113.35	10.75	.0948	−1.0232	−3.57
4:1	111.70	12.40	.1110	−0.9547	−4.76
6:1	99.70	15.10	.1513	−0.8202	−4.76
10:1	103.70	20.40	.1955	−0.7088	+2.38
15:1	100.70	23.40	.2325	−0.6336	+21.4
20:1	95.60	28.50	.2980	−0.5258	+33.4

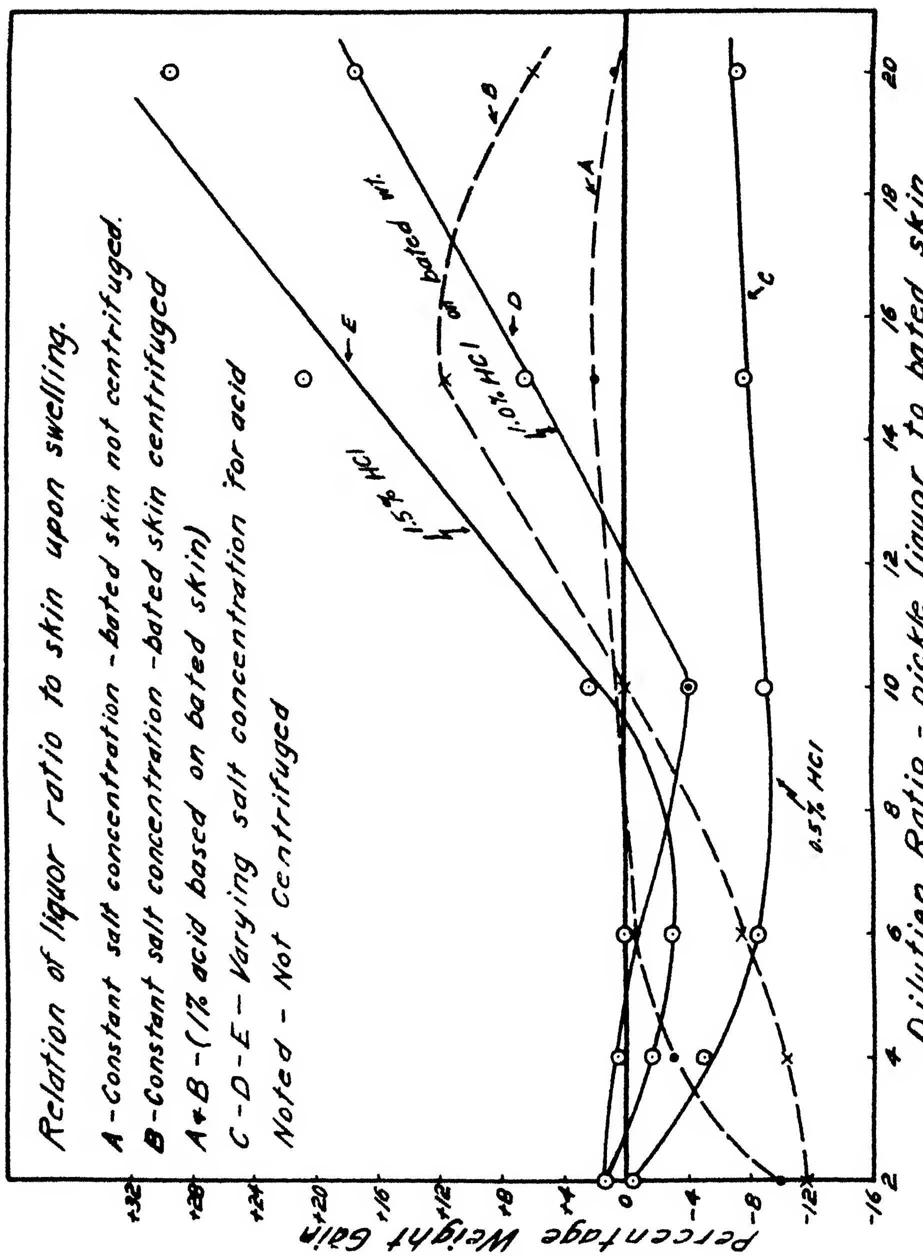
determined that the sulfuric acid-sodium chloride system is preferable in practically all respects to the one employing hydrochloric acid.

The Effect of Volume Ratio

In their original studies, Theis and Goetz had employed a volume ratio of 10 parts of pickle liquor to one of bated skin. Since in actual practice the volume ratio may vary within wide limits, depending upon whether wheel or paddle pickling is employed, Theis and Serfass[18] made a detailed investigation dealing with this factor.

For their experimental work, green salted hides were soaked for 24 hours, and then limed for five days in straight lime liquor. The hide was then unhaired, well fleshed and bated for one hour with Oropon. The bated skin was then cut into cubes of ¼″ edge and was then used for the pickling work. Samples of 45 grams were placed in 100-ml graduates, which in turn were filled to the mark with water; thus the volume of the cubes was measured. These weighed samples were then placed in the various pickle solutions. After pickling for 24 hours, the samples were again weighed and the change in volume measured. The residual pickle liquor was tested for pH value, salt and acid concentration.

Three series of pickle liquors were made. Each of the liquors contained 30 grams of salt for each 100 grams of bated skin, and in each series the acid was varied from 0.5 to 1.5 per cent hydrochloric acid. The volume ratios were varied from 2 to 1 to 20 to 1. The data obtained are shown in Tables 132 to 136 and in the various figures.



Relation of liquor ratio to skin upon swelling.
A - Constant salt concentration - bated skin not centrifuged.
B - Constant salt concentration - bated skin centrifuged
A + B - (1% acid based on bated skin)
C - D - E - Varying salt concentration for acid
Noted - Not Centrifuged
1.5% HCl
1.0% HCl on bated wt.
0.5% HCl
Dilution Ratio - pickle liquor to bated skin
Percentage Weight Gain

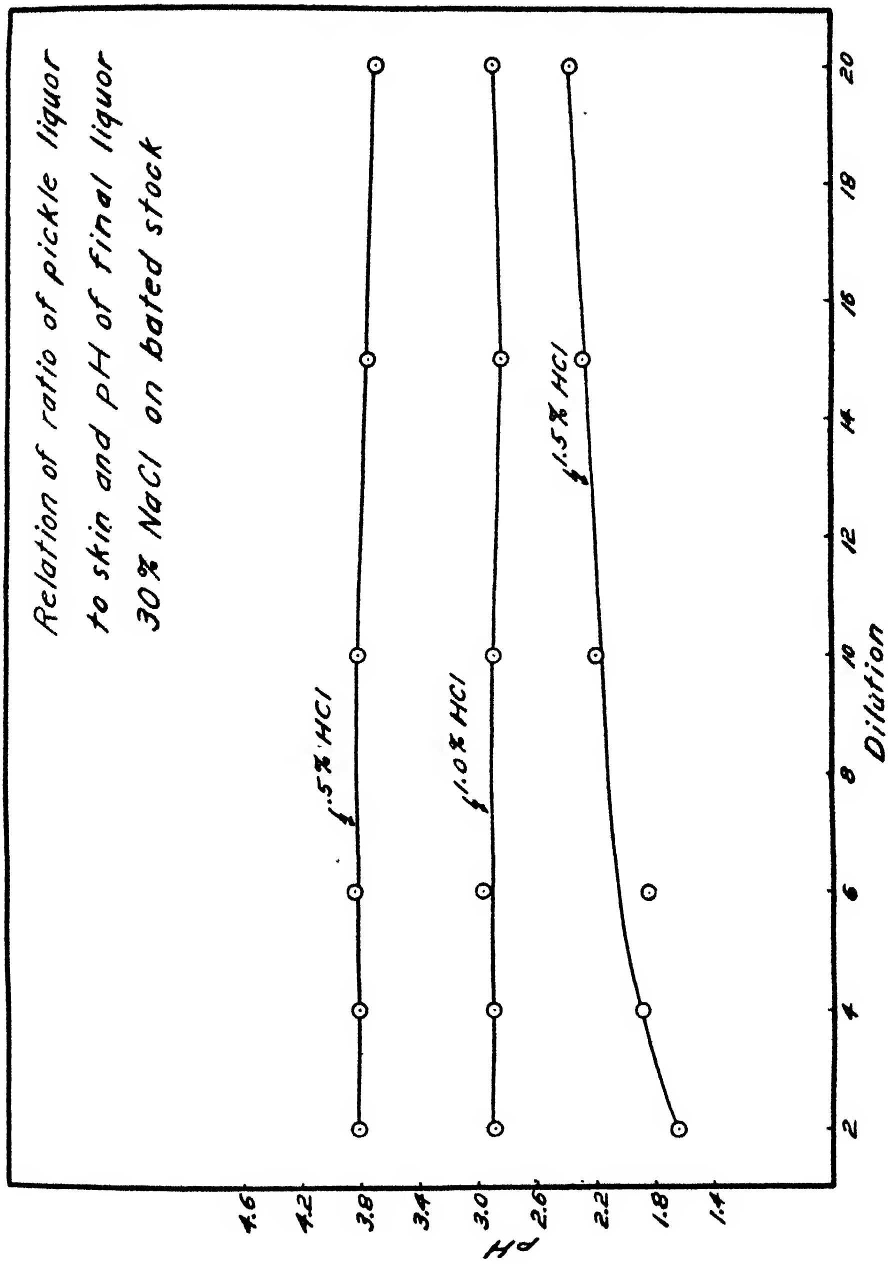

Relation of ratio of pickle liquor
to skin and pH of final liquor
30% NaCl on bated stock
.5% HCl
1.0% HCl
1.5% HCl
Dilution
pH
4.6
4.2
3.8
3.4
3.0
2.6
2.2
1.8
1.4
2
4
6
8
10
12
14
16
18
20

Figure 89

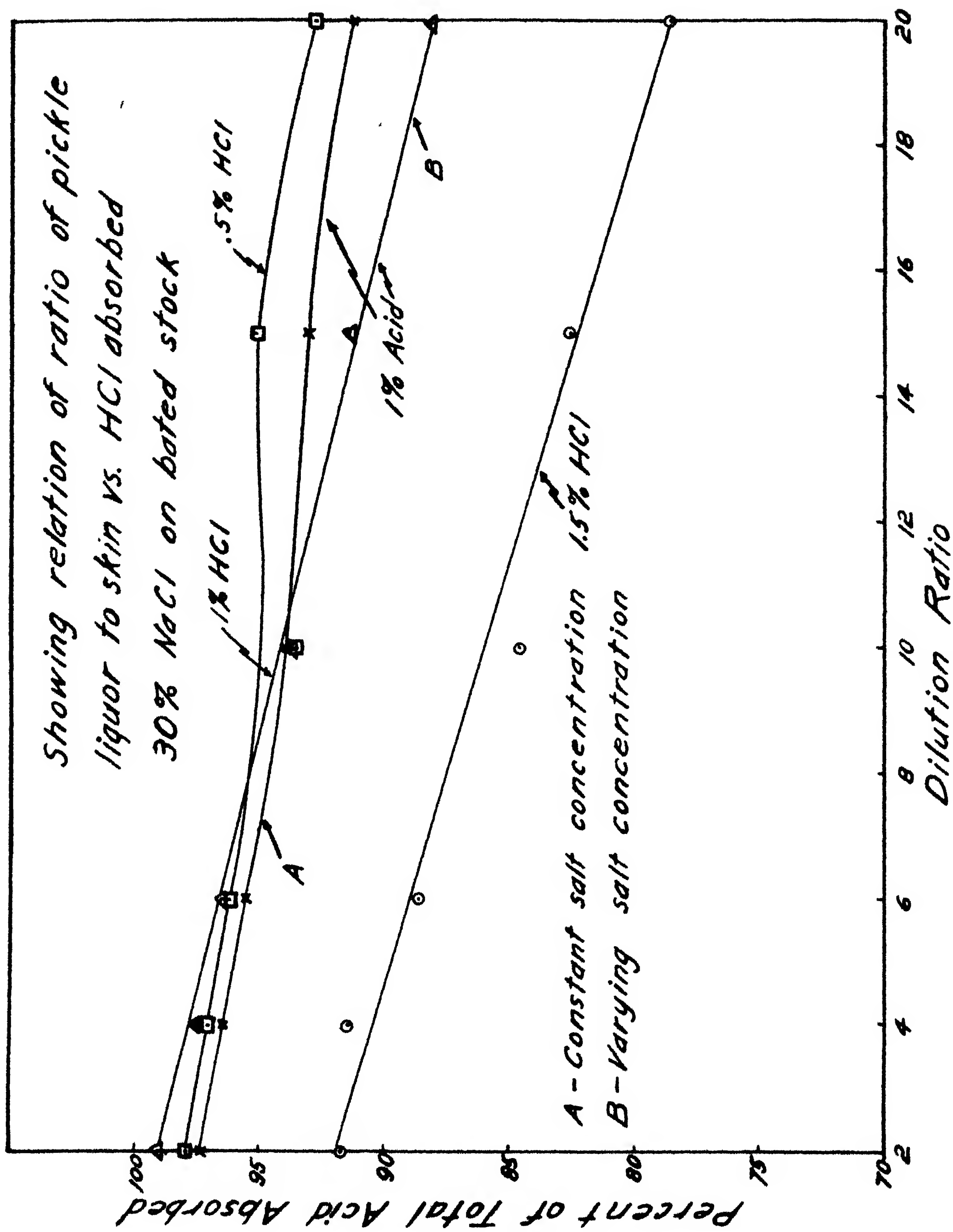

Showing relation of ratio of pickle
liquor to skin vs. HCl absorbed
30% NaCl on bated stock
.5% HCl
1% HCl
1% Acid
1.5% HCl
A
B
A - Constant salt concentration
B - Varying salt concentration
Dilution Ratio
Percent of Total Acid Absorbed
100
95
90
85
80
75
70
2
4
6
8
10
12
14
16
18
20

Figure 90

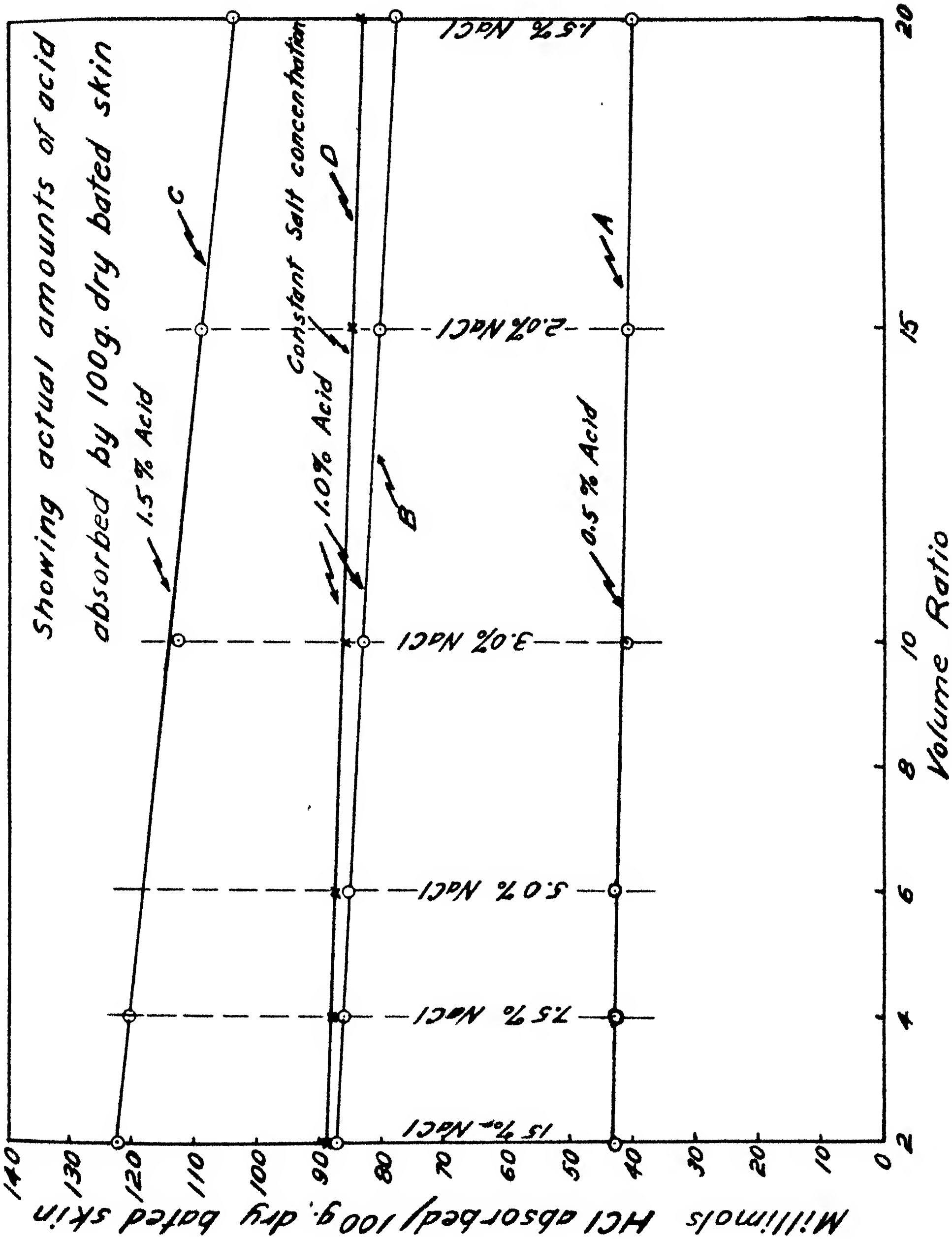
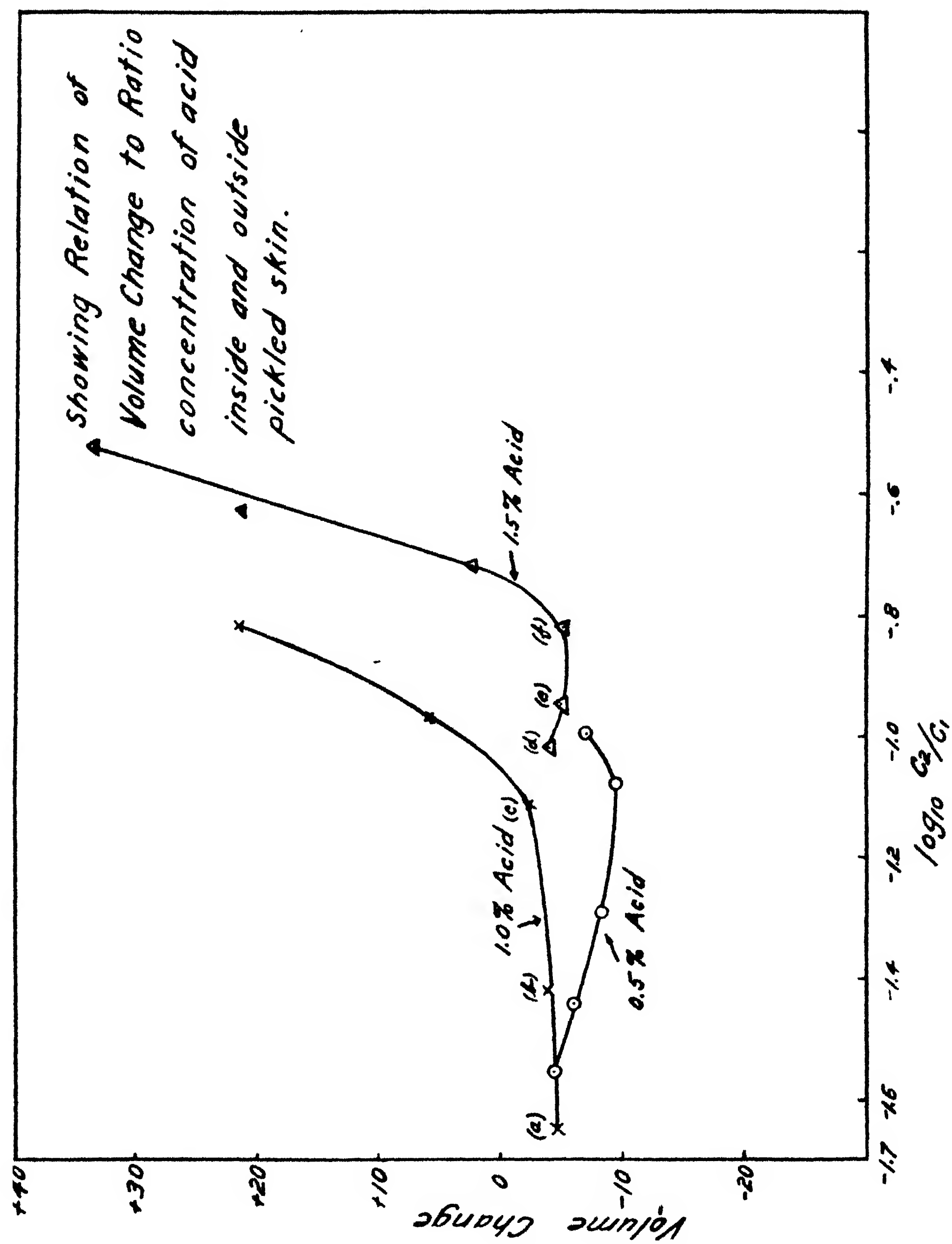
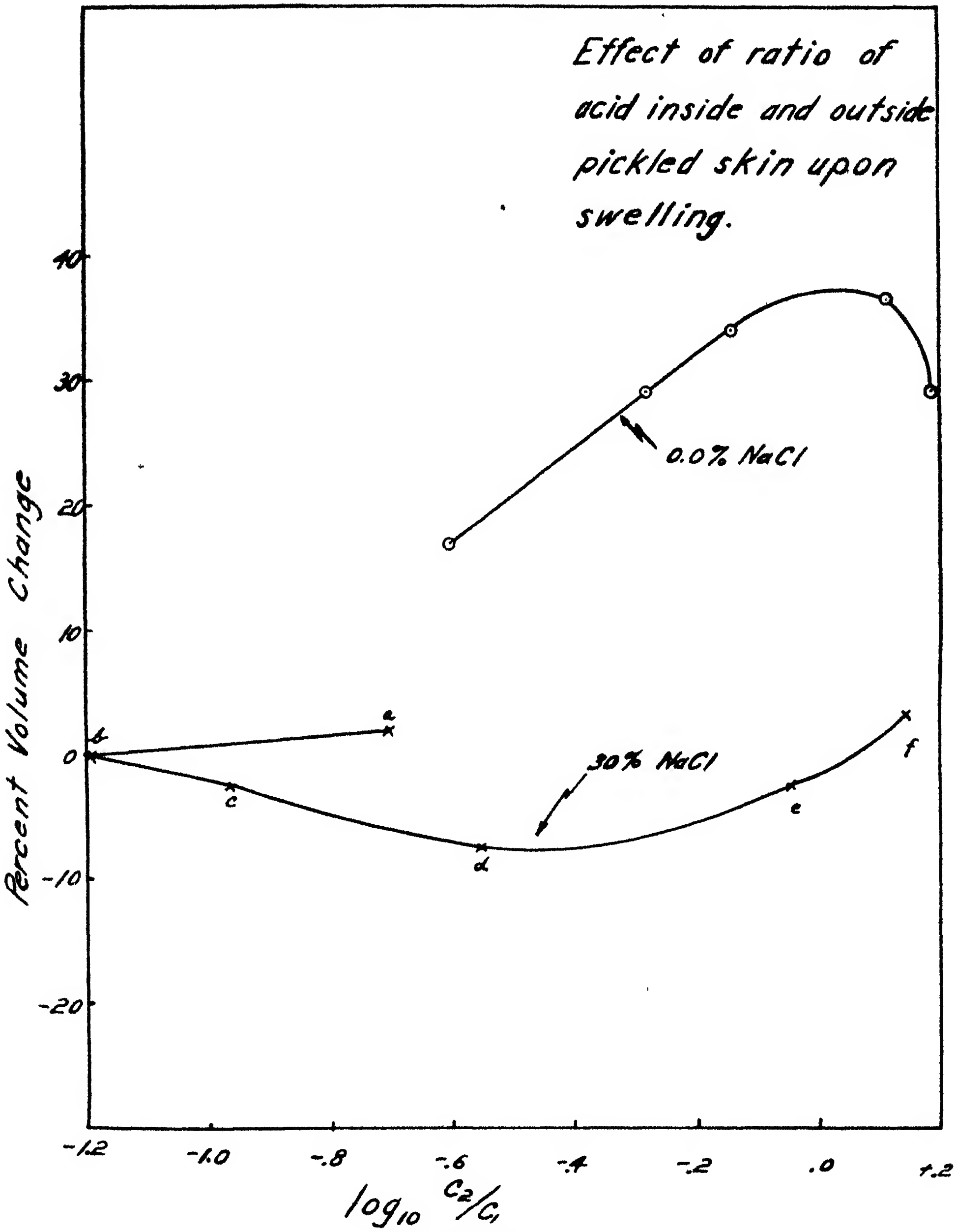

Showing actual amounts of acid
absorbed by 100g. dry bated skin
1.5% Acid
1.0% Acid
0.5% Acid
Constant Salt concentration
C
D
B
A
1.5% NaCl
2.0% NaCl
3.0% NaCl
5.0% NaCl
7.5% NaCl
15% NaCl
Millimols HCl absorbed/100g. dry bated skin
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
2
4
6
8
10
15
20
Volume Ratio

Figure 91



Showing Relation of Volume Change to Ratio concentration of acid inside and outside pickled skin.
1.5% Acid
1.0% Acid
0.5% Acid
(a)
(b)
(c)
(d)
(e)
(f)
+40
+30
+20
+10
0
-10
-20
Volume Change
-1.7
-1.6
-1.4
-1.2
-1.0
-.8
-.6
-.4
$\log_{10} C_2/C_1$

Figure 92



Effect of ratio of acid inside and outside pickled skin upon swelling.
0.0% NaCl
30% NaCl
Percent Volume Change
40
30
20
10
0
-10
-20
a
b
c
d
e
f
-1.2
-1.0
-.8
-.6
-.4
-.2
.0
+.2
$\log_{10} C_2/C_1$

Figure 93

Figure 88 shows the effect of volume ratio upon the swelling of the skin during pickling. Here it is seen that for one-half per cent acid the skin during pickling loses weight until a ratio of 10 : 1 is reached, at which point an equilibrium is apparently reached. In the case of one per cent acid there is a steady decrease in swelling (weight change) until a volume ratio of 10 : 1 is reached. As the volume ratio increases there is then a steady increase for swelling. For $1\frac{1}{2}$ per cent acid a decrease in swelling occurs up to and including a volume ratio of 6 : 1; then as the volume ratio increases there is a sharp and steady increase in swelling. Figure 89 shows the equilibrium pH value of the pickles plotted versus volume ratio. This figure is rather interesting, as it indicates that, regardless of the volume ratio (and therefore a varying acid and salt concentration), 0.5 per cent and 1.0 per cent acid concentrations (based on the bated skin weight) do not change with regard to equilibrium pH value. In the case of the 1.5 per cent acid however, the equilibrium pH increases steadily over the entire dilution scale.

Figure 90 shows the percentage of the total initially added acid which is absorbed by the skin, and Figure 91 shows the exact amounts of acid absorbed during pickling. It is interesting to note for the 0.5 per cent acid series that in practically all volume ratios 95 per cent of the acid is absorbed, and for the 1.0 per cent acid series 90 per cent and over is absorbed. For the 1.5 per cent series, the efficiency is not so great, the values varying between 77 and 93 per cent absorbed. Figure 91 indicates that the ratio change has little effect upon the acid absorption. For the series of 1.0 and 1.5 per cent acid there is a slight decrease in acid absorption with increasing dilution.

If from the millimols of acid absorbed by 100 grams of dry bated skin is substracted 8.5 (millimols used in reacting with Ca^{++}) we have the value of acid actually absorbed by the skin proteins, which we shall call C_1. The millimols of acid remaining in the residual solution we shall call C_2. If the ratio of C_2/C_1 is taken and the logarithm of the ratio plotted against volume change, we obtain curves as shown in Figure 92.

This figure presents an interesting picture in that it supports the suggestion made repeatedly by Theis and Goetz, namely, that the Donnan Equilibria theory adequately explains many of the reactions taking place during the pickling process. A study of the data shows, for the 0.5 per cent acid series, a gradual decrease in swelling results as the volume ratio is increased, or as C_2 approaches C_1. In other words, the effect of the initially added acid upon equilibrium is practically all within the skin; as the initial solution, both acid and salt, becomes more dilute, the salt has more effect than the acid, and negative swelling results. In the 1.0 per cent acid series, however, because of the acid and salt concentration at the dilutions (a), (b) and (c), the acid inside the skin remains practically the same as does the outside solution. Beyond (c), however, the acid concentration inside the skin decreases and that

outside increases, resulting in a flow from the outside solution into the skin; this is indicated by the upward trend of the curve. For the 1.5 per cent acid series (d), (e) and (f) have the same swelling because of values for C_2/C_1. Beyond (f), there is a decided drop for C_1 and thus an increase for C_2, resulting in flow of solution into the skin, as shown by great swelling. These data as a whole substantiate the use of the Donnan Membrane Equilibrium theory as applied to pickling of animal skin.

In the studies just given, Theis and Serfass varied the ratio of pickle liquor to bated skin from 2 to 1 to 20 to 1, but allowed the salt and acid to vary with the dilution. In tanning practice, any ratio from 4 : 1 to 10 : 1 is used, and it is common practice to use a pickle liquor of standard specific gravity. .This gravity may thus represent a salt concentration of from 6 to 14 per cent. For this reason, Theis and Serfass repeated certain of their experiments, employing a 6 per cent salt solution regardless of dilution, but used an acid concentration of 1 per cent based upon bated skin weight. In all the former work the bated skin, after being cut into small cubes, was blotted between towels to remove surface liquor before weighing. This procedure was again followed after removal from the pickle liquor. The work given in this section of the paper was performed in this way; but, in addition, another set was centrifuged for 10 minutes after bating and before weighing the individual samples, after pickling, and before weighing. Thus the volume change was determined. The surface liquor was removed by centrifuging, as was some of the liquor held loosely between the skin fibers. Data for these experiments are given in Table 135 and in Figures 88 to 91.

Figure 91, curves d and c shows, for both the centrifuged and uncentrifuged series, that the acid absorbed by the skin decreases slightly with dilution. This decrease is of about the same order as was the case when the salt concentration varied (curve b). Curve d shows that the acid absorption is slightly greater for a constant salt concentration. This is to be expected at the greater dilutions, since salt causes an increased absorption of acid by the skin. Curve a in Figure 90 shows this same effect. In the lower dilutions or ratios the percentage of total acid absorbed is less, but at greater ratio the percentage absorption is greater because of the higher salt centration (6 per cent constant salt concentration), as represented by Curve a (compare with Curve b). Curves a and b in Figure 88 show the swelling taking place as the ratio of pickle liquor to bated skin is varied with a constant salt concentration. Upon comparing Curve a with Curve d, it is seen that the constant salt concentration retards swelling at the higher ratios but at lower ratios the swelling is greater. Curve b represents the series in which the bated and pickled skin was centrifuged for 15 minutes before weighing, and as a consequence of this treatment the trend of the curve is somewhat different from that of Curve a. During centrifuging of the bated skin, much of the solution loosely held within

Table 137. 1.5 Per Cent HCl and Varying Concentrations of NaCl.

% NaCl solution	% Total NaCl absorbed	% NaCl absorbed by skin	% of Total acid absorbed	% Wt gain in Pickle
0	0.0	0.00	90.6	45.0
2.5	14.4	1.44	92.8	14.6
5.0	15.6	3.12	93.5	−15.8
7.5	18.8	5.64	95.0	−11.4
10.0	18.4	7.34	96.3	−15.4
15.0	15.2	9.10	96.3	− 9.6
	1.5 Per Cent H_2SO_4 and Varying Concentrations of NaCl			
0	0.00	0.00	94.3	+24.4
2.5	10.00	1.00	95.5	−11.2
5.0	11.10	2.22	96.0	−11.0
7.5	10.53	3.16	95.1	− 8.6
10.0	13.00	5.20	97.0	− 8.1
15.0	24.83	14.90	98.3	− 8.2
	1.5 Per Cent Phosphoric Acid and Varying Concentrations of NaCl			
0	0.00	0.00	61.2	+26.2
2.5	21.60	2.16	56.1	+ 0.8
5.0	22.00	4.40	56.0	+ 0.2
7.5	22.20	6.66	57.0	+ 3.0
10.0	23.30	9.32	59.8	+ 2.0
15.0	22.07	13.24	62.8	+ 9.6
	1.5 Per Cent Acetic Acid and Varying Concentrations of NaCl			
0	0.00	0.00	74.6	− 8.6
2.5	22.00	2.20	82.2	+ 3.8
5.0	23.00	4.60	86.7	+10.8
7.5	20.93	6.30	85.6	+ 9.2
10.0	20.90	8.40	86.7	+ 4.2
15.0	20.00	12.00	89.4	− 1.8
	1.5 Per Cent Oxalic Acid and Varying Concentrations of NaCl			
0	0.00	0.00	98.4	+48.2
2.5	22.00	2.20	93.8	−21.6
5.0	21.20	4.24	95.5	− 9.4
7.5	——	——	95.0	− 7.8
10.0	19.50	7.80	95.0	− 9.2
15.0	20.30	12.18	91.8	− 6.8

the skin was removed. This treatment resulted in a greater take-up of salt and water upon placing in the pickle liquor, thus reducing the salt concentration of the external solution. At the higher dilution ratios greater swelling resulted.

Various Acid-Salt Systems

Theis and Serfass[19] in 1935 investigated the pickling effect of such acids as phosphoric, acetic and oxalic, used in conjunction with sodium chloride. Their experimental technique was as follows: bated skin was placed in a pickle solution in the ratio of 1 part skin to 4 parts pickle solution; the pickle solutions were made 1.5 per cent acid, based upon the bated skin weight and the salt concentration varied from 0 to 15 per cent based upon the solution; the acids used were hydrochloric, sulfuric, phosphoric, acetic, and oxalic.

The pickling period was 24 hours at 20°. The data obtained are shown in Table 137 and Figure 94.

Figure 94 shows the effect of various acid-salt pickles upon swelling of bated skin during pickling. It would appear that each acid affects swelling differently and each has its own characteristic type of effect. The curves for hydrochloric and sulfuric acid are similar in trend, but those for phos-

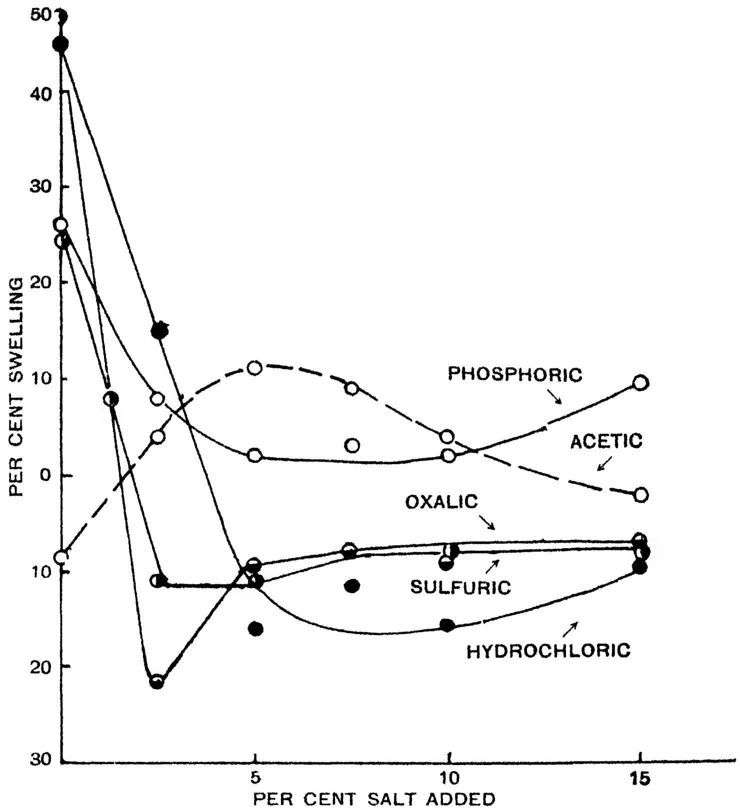


Figure 94

phoric, acetic and oxalic are entirely different. This is undoubtedly due to the different amounts of the respective acid absorbed by the skin. In the case of the hydrochloric acid-salt pickle, we have a common ion effect, which is not the case in the other pickles used. When 1.5 per cent of the several acids is employed it is found that hydrochloric acid and sulfuric acid are almost completely absorbed for all concentrations of salt. Phosphoric acid is less than 66 per cent absorbed at any salt concentration. The absorption

of acetic acid increases with the salt content of the pickle, and the absorption of oxalic acid decreases with increasing salt concentration.

The Donnan Equation

Theis and his students have repeatedly claimed that the effect of salt upon acid absorption can readily be explained by a consideration of adsorptive phenomena or the Donnan Equilibria.

When the Donnan theory is used for explaining the effect of salt upon acid absorption by animal skin, we are accepting an ideal case of a perfect semi-permeable membrane and not necessarily a chemical combination between the diffusible and non-diffusible ions or molecules. With hide or skin the so-called membrane effect is not even as perfect as that obtained with gelatin; furthermore, there is a combination of a very complex order. Jordan Lloyd, while not disputing the validity of the Procter-Wilson theory of swelling, points out that it should be modified in the case of structured proteins. She maintains that the Donnan equations were worked out for diffusion into such a volume that surface forces might be neglected; that for structured proteins the diffusion is into a set of capillary tubes; and that the charged centers and other linkages of the protein undoubtedly play an important part in the swelling phenomenon. Therefore, to apply the Donnan theory to skin protein in the same manner as that for the soluble proteins is to change from a more or less quantitative method to one of qualitative nature. Therefore, to explain acid absorption and swelling of skin by the use of the Donnan equation, we must first consider it as an ideal case. In applying this theory, it is assumed that the reader is familiar with the principle of this theory, since it has been explained in detail in Volume 1 of the second edition of this monograph.

Let $x = (H^+) = (Cl^-)$ in the external solution and
$y = (H^+)$ held within the skin
$z =$ (Hide protein combined with H^+)
$y + z = (Cl^-)$

then

$x^2 = y\,(y + z)$ or
$2x < 2y + z$ or
$2x + e = 2y + z$ when
$e =$ excess of concentration of diffusible ions inside the skin over the external phase or to set up equilibrium.

Initial State		Equilibrium State	
Collagen	$H^+ + Cl^-$	$z =$ (collagen H^+) $y = H^+$ $y + z = Cl^-$	$(H^+) = (Cl^-) = x$
		(1)	(2)

Thus

$$\frac{(Cl^-)_1}{(Cl^-)_2} = \frac{[(H^+) + (Cl^-)]_1}{[(H^+) + (Cl^-)]_2}$$

In the example above $F_{t.p.} = 0$. Therefore, the work required to transport 1 gram mol of Cl^- from $2 \rightarrow 1$ is

$$F = RT \ln \frac{(Cl^-)_1}{(Cl^-)_2} = RT \ln \frac{(H^+)(Cl^-)_1}{(H^+)(Cl^-)_2}$$

When NaCl is added to (2) and the system is permitted to come to equilibrium, the ratio of Cl^- ions will be the same as before, *i.e.*, $F = 0$. Since $(Cl^-)_2$ has increased by the addition of NaCl, $(Cl^-)_1$ must also increase to maintain the same ratio at the state of equilibrium. This increase in $(Cl^-)_2$ is caused by the diffusion of hydrochloric acid into the hide. The effect of the distribution of ions due to the addition of w mols of salt and the diffusion of a mols of acid is therefore

Initial State

(1)	(2)
$z = (\text{Collagen } H^+) = (Cl^-)$ $y = (H^+) = (Cl^-)$	$(H^+) = (Cl^-) = x$ $(Na^+) = (Cl^-) = w$

$$P_1 < P_2$$

Equilibrium State

(1)	(2)
$a = (H^+) = (Cl^-)$ $z = (\text{Collagen } H^+) = (Cl^-)$ $y = (H^+) = (Cl^-)$	$(H^+) = (Cl^-) = x - a$ $(Na^+) = (Cl^-) = w$

$$P_1 = P_2$$

Experimental findings have proved that the addition of NaCl to the acid pickle caused up to a 33 per cent increase in acid absorbed by the hide, which fact substantiates the theory outlined above.

If, in the system hide (collagen)-hydrochloric acid, we determine the ratio: concentration acid outside the hide (C_2) to concentration acid inside the hide (C_1), then take the logarithm of this ratio and plot this value against swelling or volume change, we are able to postulate that whenever $C_2 < C_1$ swelling occurs, when $C_2 = C_1$ maximum swelling results and when $C_2 > C_1$ swelling decreases. At the point $C_2 = C_1$ the greatest excess of diffusible ions results; and thus further addition of acid, making $C_2 > C_1$, results in a decrease in diffusible ions with subsequent decrease in swelling. *We may then define swelling as due to the flow of liquid from the less concentrated solution to the more concentrated solution either within or outside the skin as the case may be.*

Referring to Figure 93, it can readily be seen for the case of 0 per cent salt that the swelling increases for all data when $C_2 < C_1$, or when the flow of solution is into the skin, but when $C_2 > C_1$ a decrease in swelling results. When

NaCl is added to the system an entirely different type of curve results. At point (a) there are 12 millimols HCl inside the skin and only 0.7 millimol and 50.3 millimols NaCl outside; at point (b), 24.4 millimols HCl inside and only 1 millimol HCl outside; at point (c), 58.2 millimols HCl inside and only 5.41 millimols outside; at point (d) 101.5 millimols inside and 25.38 millimols outside; at points (e) and (f), 105.2 and 111.1 millimols inside and 84.47 and 138.62 millimols outside. The reason for the shift of (b) to the left of (a) is due to the fact that the salt is causing so much HCl to penetrate inside, thus causing C_1 to be very large in respect to C_2. The millimols HCl in the external solution for both (a) and (b) is practically the same, showing that the addition of NaCl caused this large amount of penetration. However, at (c), though the penetration inside the skin was much greater than at (b), there was a concentration in the outside solution 5 times as great. At (d) the penetration inside the skin attained an approximate equilibrium, while the concentration outside was 36 times greater than at (a). In all cases, (a), (b), (c) and (d), solution was flowing from inside the hide to the more concentrated solution outside, thus giving negative swelling. At (e) and (f) the acid concentration inside was but little greater than at (d), but the outside concentration had increased to 3.5 to 5.5 times that of (d), and as shown by the trend of the curve, the flow of liquid now tends from the outside into the skin. Figure 71 also substantiates the explanation given above.

The authors are well aware that in consideration of the Donnan theory as applied to acid absorption and swelling of animal skin it is necessary to modify the assumptions made by Procter and Wilson and later by Loeb for gelatin-acid systems in translating them to the fibrous and structured protein systems. Such modifications having been made, the Donnan Membrane Equilibria Theory does explain, in its own particular way, many of the phenomena found in the pickling process.

The Bound and Free Electrolyte

When bated skin is placed in a pickle solution, acid and salt diffuse into the skin; part of this acid is fixed by certain reacting groups of the collagen and the remaining fraction of the acid is loosely held within the skin. At the usual pH values prevailing during pickling, 1.5 to 3.0, it is doubtful if any sodium chloride, as such, is actually bound. However, acid is bound, and the amount fixed by the skin protein is that normally obtaining for the equilibrium pH value of that pickle solution. In the consideration of subsequent mineral tannage, both types of acid are important, since both the acid chemically bound and that mechanically held influence the subsequent tannage. In the studies made by Theis *et al.* during the period 1931-35, no attempt was made to differentiate between the acid bound and that which existed in the free state. (Nor did Küntzel.)

McLaughlin and Adams[7] in 1940 introduced a new technique in a study of the acid-binding of collagen. In this work, these investigators pressed the acid-treated collagen material at 5000 pounds per square inch and held that such pressing removed, for practical purposes, all the mechanically held acid. In their conclusions, they pointed out that collagen treated with sulfuric acid in the range of 4 to 19 per cent gave a straight line when the log of acid fixed was plotted versus the log of acid unfixed. From these data such a conclusion is completely valid in the equilibrium pH range of 0.85 to 2.05. At pH ranges below or above this, the conclusion does not hold good. The data relative to the investigation of McLaughlin and Adams have already been given in some detail in Chapter 4.

Their work was significant, because (1) it brought to the attention of the protein chemist and the leather chemist a unique means of ridding the tissue of free water and free electrolyte; and (2) by the use of this method, new and important facts have been discovered.

Theis and Jacoby[16] in 1941 made a study of the acid bound by collagen from three different pickle solutions: (a) sulfuric acid, (b) sulfuric acid-normal sodium chloride and (c) sulfuric acid-normal sodium sulfate. In each of these types the pH value was varied between 0.4 and 7.3. A 24-hour pickling period at 25° was employed.

After pickling, the skin was removed, pressed twice at 10,000 pounds per square inch, air-dried, and then ground in a Wiley mill to a 60-mesh powder. The material so prepared was then analyzed for fixed H^+ ion and nitrogen. The methods used are given in detail in Chapter 4. Figure 95 shows the data obtained for the sulfuric acid, no salt, system. This figure is shown in three parts: A, the grams of sulfuric acid fixed by one gram of hide substance; B, the ml of $0.1N$ H_2SO_4 bound by one gram of hide substance; and C, the ml $0.1M$ H_2SO_4 bound by one gram of hide substance. The data taken are plotted in each case against the equilibrium pH value of the pickle solutions. This method of plotting in reality gives us an acid titration curve of the protein in question. The acid titration curve of Figure 95 shows that this acid curve should be divided into three distinct zones; the first, pH 0.5 to about 1.0, which shows great acid take-up; the second, pH 1 to pH 2.5, in which the log acid fixed versus log acid unfixed is a straight line indicative of adsorption; and third, the range pH 2.5 through the isoelectric zone, which does not follow the adsorption law. Curves B and C show that at about pH 1.3 there is a tendency toward maximum acid fixation, but at pH 1.0, hydrolysis effects enter, and perhaps through simplification of the protein more acid is bound. This fact is well illustrated in the pH range 0.5 to 1.0. In the second zone the log of the fixed versus the log of unfixed acid follows a straight line. In Figure 95 the dotted line represents values obtained by McLaughlin and Adams; and it can readily be seen that the results check well.

The third zone, pH value 2.5, shows that the acid bound constantly decreases to an actual zero value at pH 6.5, thus giving an isoelectric point close to that usually obtained by cataphoretic experiments.

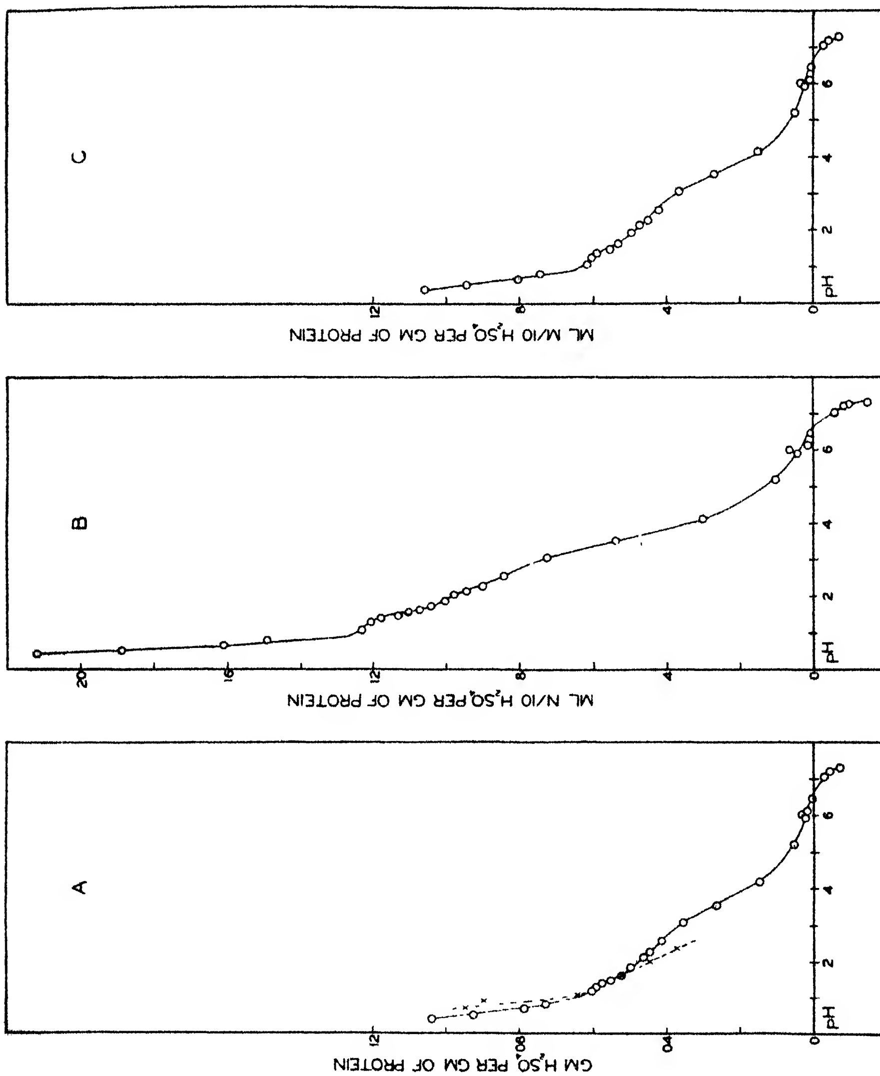


Figure 95. Showing the sulfuric acid combined with collagen when sulfuric acid alone is used for pickling. The dotted line in "A" represents data taken by McLaughlin and Adams by an entirely different method.

In the actual pickling of hides and skins, we are interested in the pH range 1.0 to 4.0. Here we see that the actual acid bound at pH 1.0 is 6.0 per cent, while at pH 4.0 this figure is approximately 2.0 per cent. These figures give

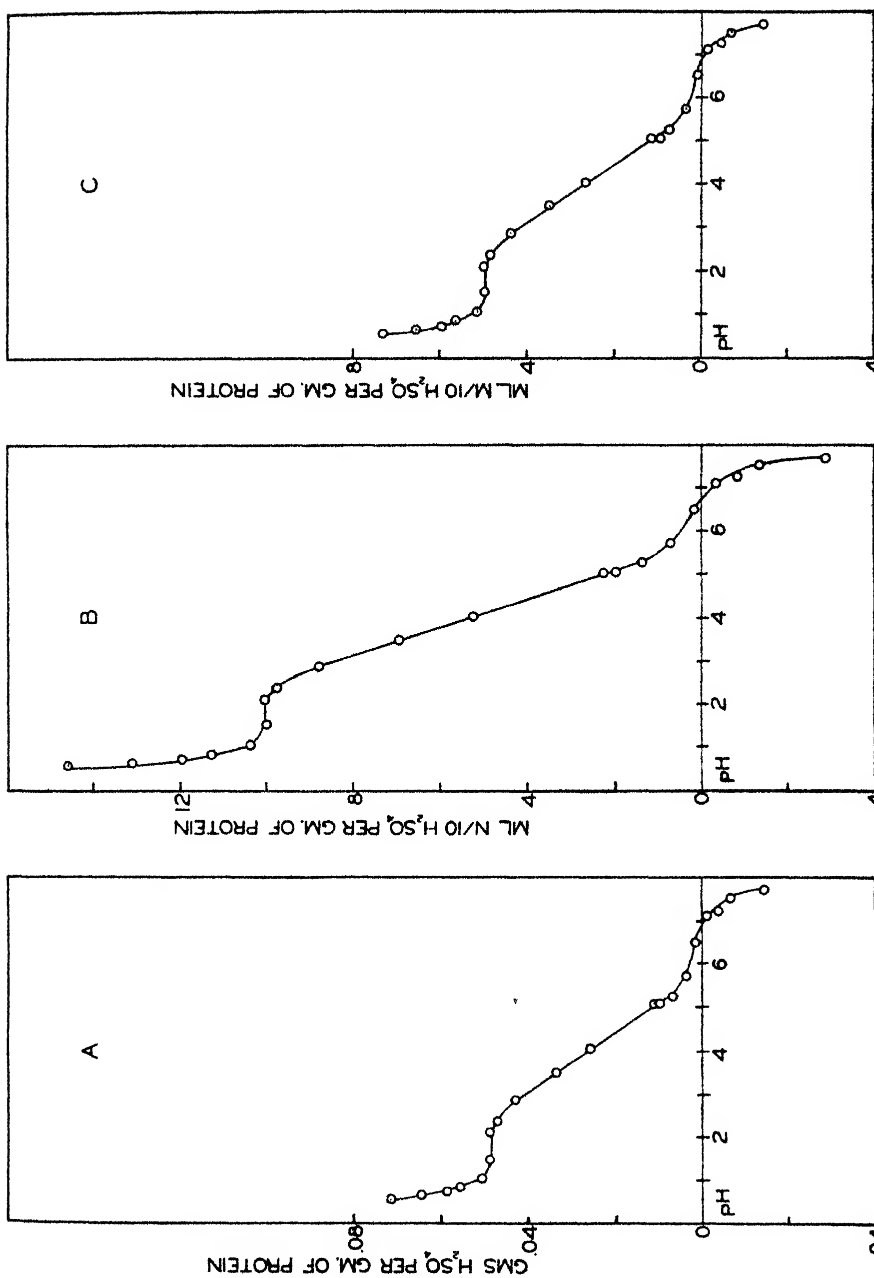


Figure 96. Showing the sulfuric acid combined with collagen in pickle solutions containing both sulfuric acid and sodium chloride (1.0 Normal).

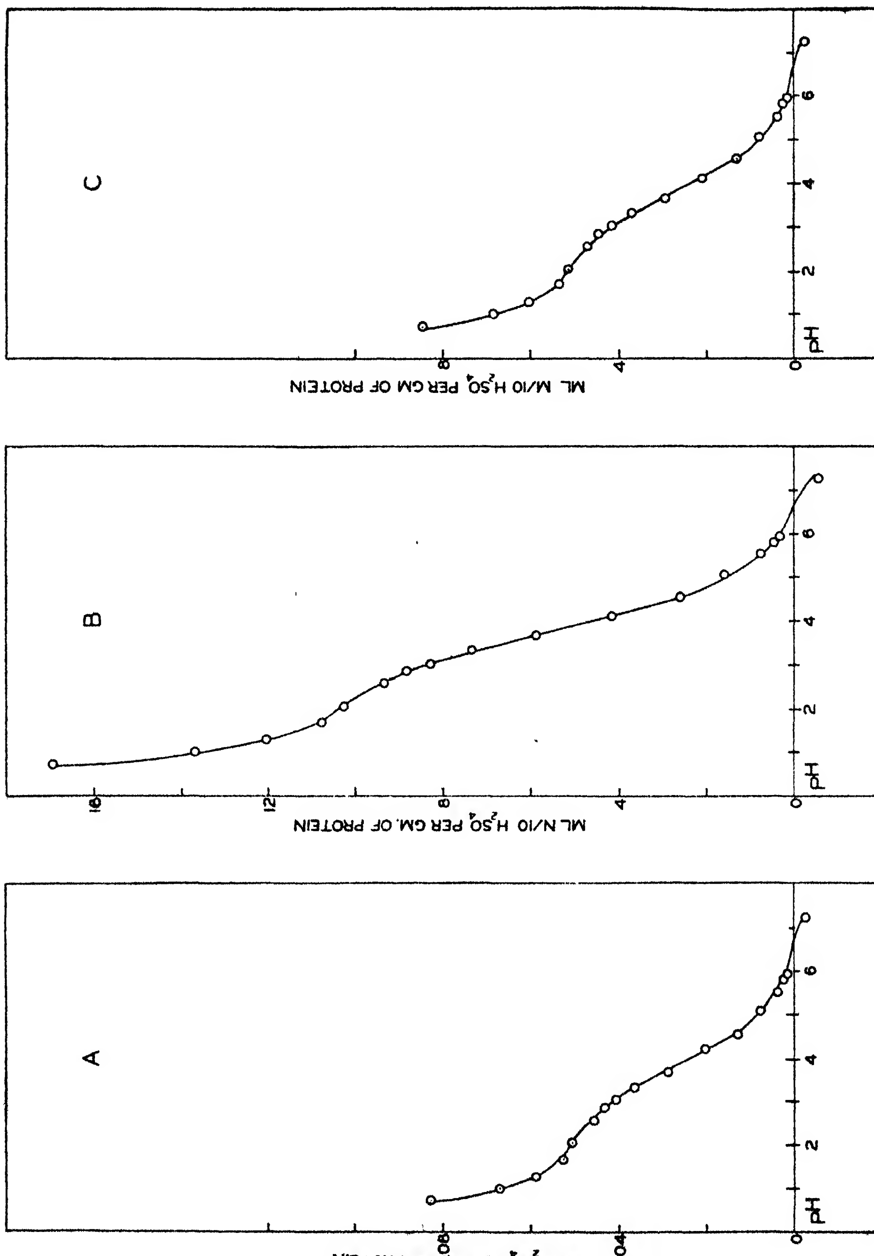


Figure 97. Showing the sulfuric acid combined with collagen in pickle solutions containing both sulfuric acid and 1.0 Normal sodium sulfate.

no idea of the acid actually held mechanically between the skin fibers; they represent only that fixed by the protein substance.

Figure 96 A, B and C, shows the data obtained from the system sulfuric acid-sodium chloride. This is the usual type of pickle utilized by the chrome tanner and should be of more interest than the first system discussed.

It was shown in Figure 95 that there was somewhat of a maximum acid fixation between pH 1.0 and 2.0. Figure 96 shows this trend in a more pronounced manner. It would therefore seem that the sodium chloride (normal solution) causes a decreased protein simplification, but its effect is not sufficient to entirely obviate it, because at pH 1.0 there is again a sharp rise in the curve. The curves given in Figure 95 again show that the isoelectric point is about pH 6.7. In the pH range 1.0 to 4.0 it is found that 5 per cent sulfuric acid is bound at pH 1.0, while only 2.6 per cent is bound at pH 4.0. Thus it appears that the NaCl has reduced the acid fixation at pH 1.0 and increased it at pH 4.0. However, it must be remembered that in a pickle made up of sulfuric acid and sodium chloride, there are present H^+, Na^+, Cl^-, HSO_4^- and $SO_4^=$ ions, and thus it is impossible to postulate directly just which anions are held by the protein molecule.

While the normal pickle system used in practice is the one of H_2SO_4--NaCl, use is often made of the system H_2SO_4—Na_2SO_4. In this case, there is a common ion, and a somewhat simpler system results. Figure 97 gives data for this system. In the very acid range, the sodium sulfate gives a very small increase in acid fixation over that given by acid alone, and in the less acid region a somewhat greater acid fixation over acid alone, but still less than when sodium chloride is present. The isoelectric point given by this system is about pH 6.7. At pH 1.0, the acid fixed is 6.7 per cent and at pH 4.0 it is 2.4 per cent. Thus we might tabulate acid fixed in the practical pickle range for the three systems as follows:

Table 138.

	Acid Fixed*		
pH Value	H_2SO_4	H_2SO_4 NaCl	H_2SO_4–Na_2SO_4
1	6.4	5.0	6.7
2	4.8	5.0	5.1
3	3.6	4.0	4.1
4	2.0	2.6	2.4

* Per cent on protein basis.

The types of curves given for the pickle systems H_2SO_4—NaCl and H_2SO_4—Na_2SO_4 are quite different. In the case of the H_2SO_4—NaCl, there is a distince flat range between pH 1.0 and 2.3 in which the acid fixed remains more or less constant. Such is not the case for the system H_2SO_4—Na_2SO_4. Since this is the actual practical range usually employed in the pickling operation, this finding may well represent the real difference found under

practical conditions of pickling between sulfuric acid-sodium sulfate and sulfuric acid-sodium chloride systems.

McLaughlin and Adams pointed out in their work dealing with the acid-binding capacity of collagen that the log unfixed acid versus log fixed acid resulted in a straight line. However, recalculation of the work of Theis and

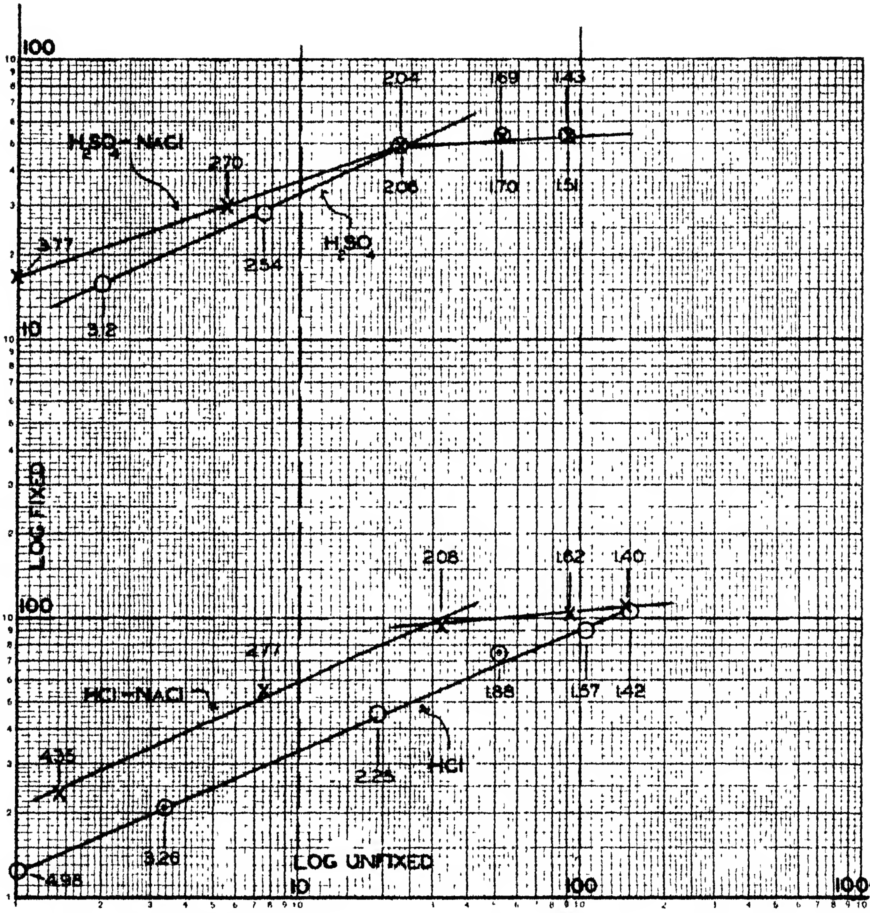


Figure 98. Indicating the relation of the acid absorbed to that remaining unabsorbed when acid alone is used and when an acid-salt pickle is used. The curves are shown in a log/log relation.

Goetz dealing with HCl—NaCl and H_2SO_4—NaCl pickles shows that even if the skin is not pressed (thus containing both collagen bound and mechanically held acid) the log of the acid held within the skin (equivalent to conditions obtaining in the actual pickling operation) plotted versus the log of the

remaining acid also results in a straight line. Figure 98 shows such recalculated data. The curves given in Figure 98 indicate some interesting trends wholly in line with the accurately determined bound acid. The curves representing HCl alone and HCl with 10 per cent NaCl show, for acid alone, that all the points from pH 1.42 to pH 4.98 fall on the same straight line, while for the HCl in the presence of NaCl such is not the case. For this particular case the straight line range is 2.08 to 4.35. Either above or below this range the acid bound and occluded does not follow the equation. This, of course, may be accounted foı by application of the Donnan Theory, in that at pH values greater than 4.35 there is not sufficient acid present in the outside solutions for a greater acid take-up, and practically all the acid has been absorbed by the skin. However, at pH values greater than 2.08 it might be said that though more acid has been absorbed by the skin in the presence of NaCl than in the presence of acid alone, the acid absorption capacity (bound and mechanically held acid) of the skin has been reached, and this point lies approximately at pH 2.0 when NaCl is present and at 1.4 when only HCl is present.

However, when H_2SO_4 and H_2SO_4—NaCl are used for pickling a somewhat different picture results, as is seen in Figure 98. In this case the straight line representing the H_2SO_4--NaCl is not even approximately parallel to the line representing the H_2SO_4. The two lines meet at a point at a pH of 2.04, representing maximum absorption of acid (bound and mechanically held); and as the pH is further decreased little or no additional absorption of acid takes place up to pH 1.4.

Since hides and skins are usually pickled in a solution of sulfuric acid and sodium chloride, and since they are usually only drained and thus contain both bound and mechanically held water, it is of practical importance to note the sulfuric acid-sodium chloride curve in Figure 98. This curve denotes that in the pH range 2.0 to 1.4 (practical pickling) the skins entering the chrome tanning bath contain the same total quantity of acid in this pH range regardless of the specific pH value of the pickling bath. The salt will of course affect the swelling and plumping of the skins.

Looking back now to Figure 97 it will be found that the actual bound acid shows fixed values in the pH range 2.1 to 1.0, indicating that both the acid fixed by the skin proteins and the acid absorbed and held mechanically appear to obey the same general law. At pH values less than 1.0, however, this axiom does not hold, since undoubtedly simplification and acid hydrolysis of the skin protein take place, as can be seen by the sharp and distinct rise in the curves in Figure 97.

However, looking at the straight lines representing HCl alone and HCl—NaCl systems, it can readily be seen that there is no flat region in the acid curve, and that all the points from pH 4.98 to pH 1.4 fit the straight line,

very unlike the sulfuric acid curve. However, the H_2SO_4—NaCl line shows that maximum acid absorption takes place at pH 2.08 and little more acid is absorbed at higher concentrations of acid. Thus it may be said that in the pH range 2.08 to 1.4, a HCl—NaCl pickle causes the pickled skins to carry into the chrome tanning liquid approximately the same amount of acid.

McLaughlin and Adams in subsequent studies (1942) investigated the effect of sodium sulfate upon sulfuric acid take-up and found that the added salt decreased the acid fixation from a given sulfuric acid solution. A detailed account of these investigations has been given in Chapter 4 and therefore will not be further discussed.

The average pickle solution used by the American tanner will consist essentially of a $0.1N$ solution of sulfuric acid made $1.0N$ with respect to sodium chloride. The average bated skin placed in such an acid-salt solution will bind some 5.0 per cent sulfuric acid at pH 1.0 and some 4.0 per cent at pH 4.0. Theis and Jacoby have recently shown that at pH 1.0, collagen does not bind alkali chlorides. However, at pH 4.0, and a sodium chloride concentration of $1.0N$, they show some actual binding of this salt. From such data, it might be suggested that little sodium chloride is fixed by the collagen from pickle solutions usually employed, and that the salt contained in the pickled skin is mechanically held.

Hydro-thermal Stability

The authors have previously discussed hydro-thermal stability, or shrinkage temperature. This term has been defined as the point at which the increasing disruptive forces exceed the diminishing cohesive forces.

In 1940, Theis and Esterly[13] studied the effect of hydrochloric acid and sodium hydroxide upon collagen, both alone and in conjunction with sodium chloride. They found that in the pH range 4.0 to 10.0, using acid or alkali with no salt, the shrinkage temperature of the collagen remains essentially constant, namely from 55° to 57°. At lower or higher pH values, the shrinkage temperature decreased. This decrease was especially noticeable on the acid side. If salt (NaCl) was added to the acid or base solutions, the effect was to increase drastically the shrinkage temperature of the collagen over the pH range 1.0 to 10.0. At pH values greater than 10.0, no real increase occurred. These data indicate that the salt in the pickle plays a predominant role. The data of Theis and Esterly are shown in Figure 32 of Chapter 5.

In 1941 Theis[12] determined the shrinkage temperature of collagen treated 24 hours with sulfuric acid, hydrochloric acid, acetic acid, formic acid and phosphoric acid. These data are shown in Table 139.

These data show that regardless of the acid employed, the shrinkage temperature of the treated collagen is approximately the same for any given pH value of the pickle acid. Theis investigated the effect of sodium chloride

Table 139. Shrinkage Temperature of Pickled Skin.*

pH of Pickle	H_2SO_4 (°C)	HCl	HAc	H_3PO_4	H·COOH
1	40.2	39.2		39.4	...
2	42.6	44.0	..	42.8	43.0
3	43.0	47.2	45.2	47.5	46.8
4	52.2	49.0	50.6	53.4	53.0

* No salt used in the pickle solutions and these pH values were maintained throughout the pickling period.

additions to sulfuric acid upon the shrinkage temperature of collagen treated with such pickle solutions. These data are shown in Table 140 and in Figure 99.

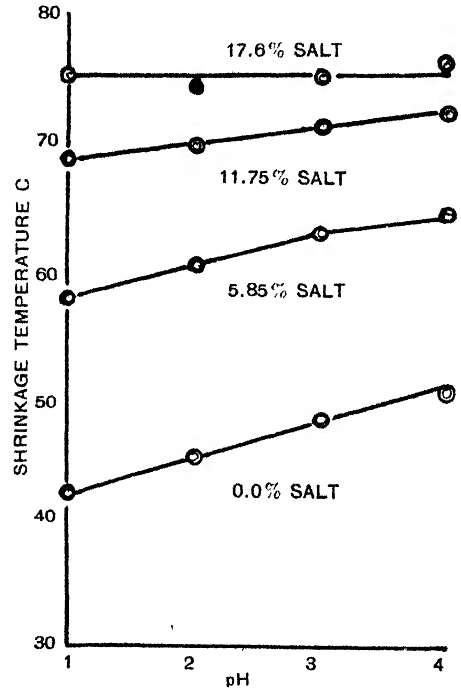


Figure 99

Table 140. Shrinkage Temperature of Pickled Skin, with H_2SO_4 and Varying Amounts of NaCl

pH of Pickle	No salt (°C)	5.85% NaCl	11.75% NaCl	17.6% NaCl
1	42.8	57.6	68.5	75.0
2	45.0	60.0	69.5	74.0
3	48.0	62.4	71.0	75.0
4	50.0	63.8	72.0	76.0

These data show for the skin pickled in such solutions: (a) that as the pH value of pickling increases, the resistance to temperature increases; (b) that this increase in temperature resistance continues as the salt concentration of the pickle is raised; and (c) that if sufficient salt is present in the acid pickle, regardless of pH value, the resistance to temperature becomes almost constant.

In 1942, Theis and Steinhardt[20] investigated the effect upon collagen of such pickle systems as: (a) HCl—NaCl; (b) HCl—$CaCl_2$; (c) $AlCl_3$—NaCl (d) $AlCl_3$—Na_2SO_4; (e) $Al_2(SO_4)_3$—NaCl; and (f) Al_2SO_4—Na_2SO_4. The data regarding the HCl—NaCl and HCl—$CaCl_2$ systems have been given in previous chapters. It was found that treating collagen with either HCl—NaCl or H_2SO_4—Na_2SO_4 solution gave it an increased resistance to temperature. However, when $CaCl_2$ replaced NaCl in the pickle, the shrinkage temperature of the treated collagen decreased sharply. In reality, the skin proteins became denatured in a somewhat similar manner to heat-denatured collagen. This work has been previously discussed and is shown in Figure 33 of Chapter 5.

Theis and his students in 1936 and 1942 made a study of various alum pickles and found that when bated skin was pickled in aluminum sulfate solutions, more Al_2O_3 than SO_3 was taken up at low concentrations, whereas at high concentrations more SO_3 than Al_2O_3 was absorbed. Addition of neutral salts to aluminum sulfate pickles tended to cause a greater absorption of Al_2O_3 than SO_3. As in the case of acid-salt pickles, the neutral salts repressed swelling. These data are shown in Figures 100 to 103.

An investigation was also made on the effect of various aluminum salt pickles upon the shrinkage temperature of collagen treated with them. The experimental technique used by Theis *et al.* is as follows.

Goat skin properly soaked, limed in a straight lime for 5 days, thoroughly washed in running water, delimed with acetic acid, again washed and then completely dehydrated with acetone was used for the experiments outlined in this paper. The dehydrated skin was placed in 100 ml of the respective salt solutions for 24 hours, being constantly agitated and kept at 20°. The shrinkage temperature of the treated skin was obtained by employing the equilibrium solution as the heat exchange medium when using the shrinkage test machine. The data obtained are shown in the following figures.

Figure 100 shows the data obtained for the systems ($AlCl_3$), and ($AlCl_3$ + NaCl). Curve A of this figure shows the shrinkage temperature of skin treated with dilute and concentrated aluminum chloride solutions—the aluminum chloride being the only salt present. It is thus seen that shrinkage temperature decreases in the concentration range 0 to 0.2 mol $AlCl_3$ and then steadily increases in the range 0.2 to 1.5 mols of salt.

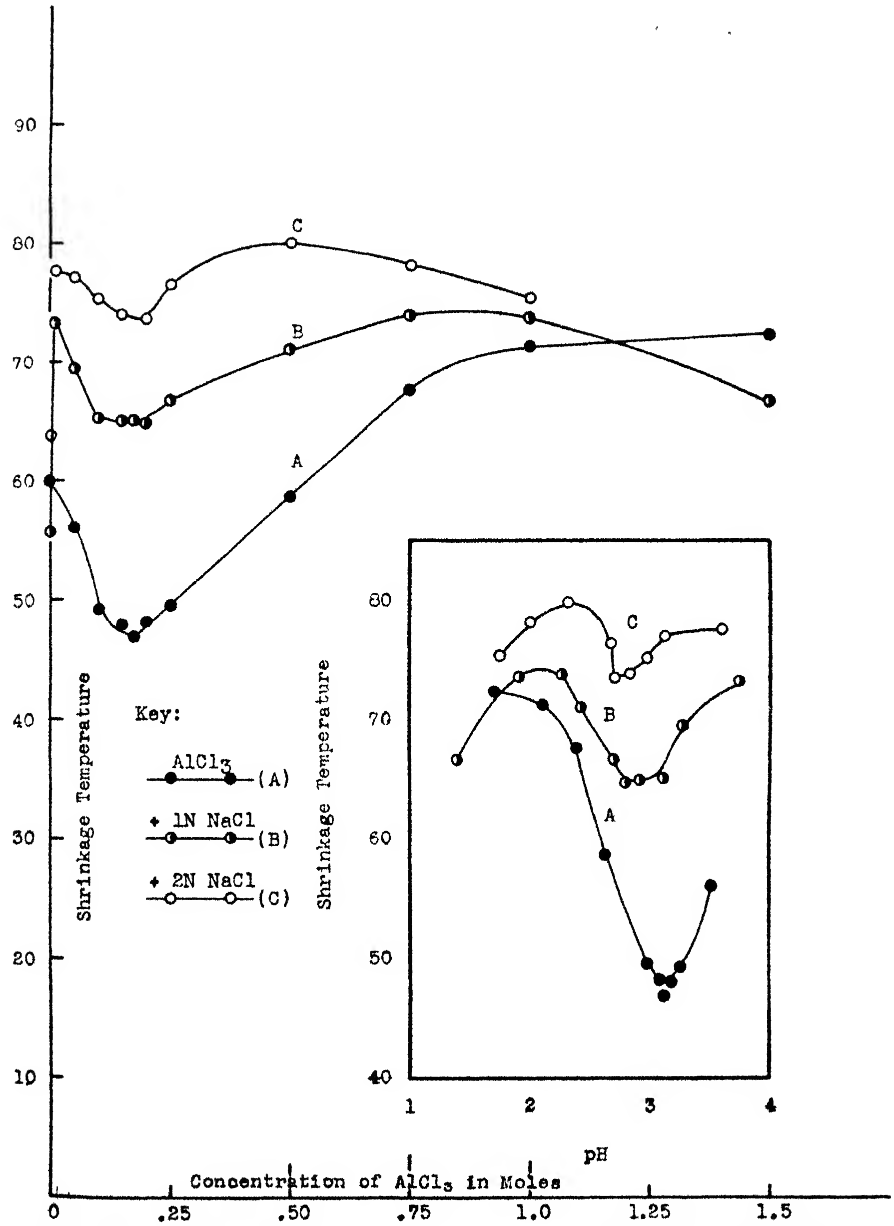


Figure 100. Effect of Aluminum chloride—sodium chloride pickles upon shrinkage temperature of treated collagen.

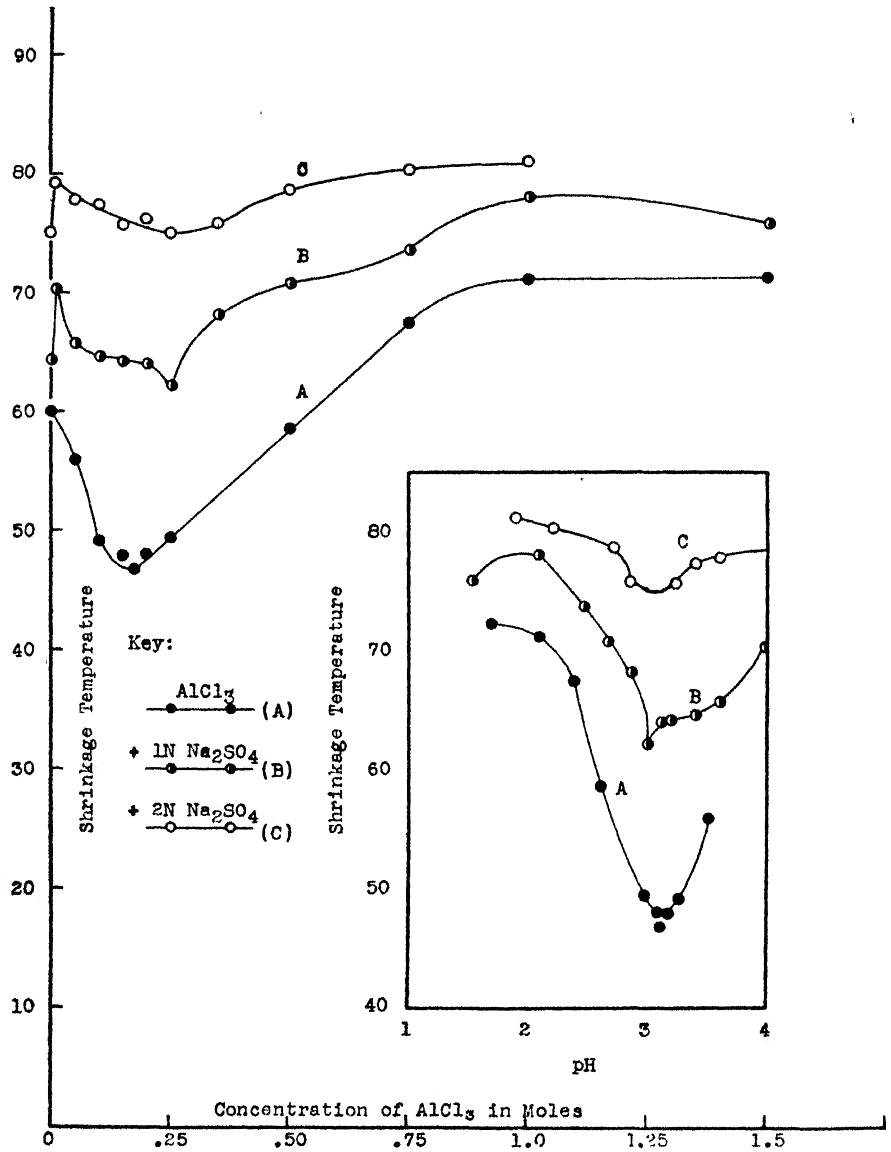


Figure 101. Effect of Aluminum chloride—sodium sulfate pickles upon shrinkage temperature of treated collagen.

In the dilute range, the aluminum chloride apparently caused a breakdown of the electrovalent linkages (salt linkages) due in all probability not only to the direct salt effect,

$$\left[\begin{array}{ccccccc} \mathrm{Cl}- & & \mathrm{Cl}- & & \mathrm{Al}+ & & \mathrm{Al}+ \\ \text{————} & \mathrm{NH_3^+} & & \mathrm{Cl}-\ \mathrm{Al}+ & & -\mathrm{OOC} & \text{————} \\ \mathrm{Cl}- & & \mathrm{Cl}- & & \mathrm{Al}+ & & \mathrm{Al}+ \end{array}\right]^{+}$$

but also to a partial back titration or breaking of the zwitterion because of the active acidic reaction of the hydrolyzable aluminum chloride, $AlCl_3 + H_2O \rightarrow AlOHCl_2 + HCl$. This causes the very drastic decrease in shrinkage temperature which the authors believe is indicative of a rapid structural change in the electrovalent link. (Note the pH-shrinkage temperature curve.) While there may possibly be some slight structural change in the carbonyl-imino linkages in this range, this breakdown is undoubtedly small and we are forced to postulate that the main decreases in cohesion forces are those of the electrovalent link. However, at molar strengths greater than 0.2, aluminum chloride acts in a somewhat similar manner to sodium chloride, causing (a) a decrease in osmotic pressure; (b) dehydration and the removal of water from the tissue, thus forcing the carbonyl-imino groups closer together; and (c) an increased structural stability as measured by the shrinkage temperature. At exactly 0.2 molar aluminum chloride two forces are exactly balanced (1) the destruction of the electrovalent linkages caused by salt effect and acidic reaction of the salt; and (2) the strengthening of the carbonyl-imino link caused by the dehydration of the tissue. At concentrations greater than 0.2 molar, the strengthening of the carbonyl-imino link becomes predominant and far overbalances the destruction of electrovalent links, thus giving rise to increased structural stability. It should be pointed out that at a molarity of 1.5, the equilibrium pH value of the aluminum chloride solution is 1.7—thus making for almost complete destruction of the electrovalent links—yet the structural stability of the protein has increased enormously. These facts strengthen our dogmatic contention that the weakening of the electrovalent links is merely reversibly incidental and that the carbonyl-imino links must be affected in order to show either drastic decrease or increase in the structural stability of collagen.

Curves B and C in Figure 100 give data for aluminum chloride solutions made 1 and 2 normal, respectively, with respect to sodium chloride. These curves are of practical importance because mixtures of aluminum salts and sodium chloride are often used in processing hides, skins and furs; they show rather strikingly that the addition of sodium chloride to the aluminum chloride solutions materially changes the effect of the latter on the skin,

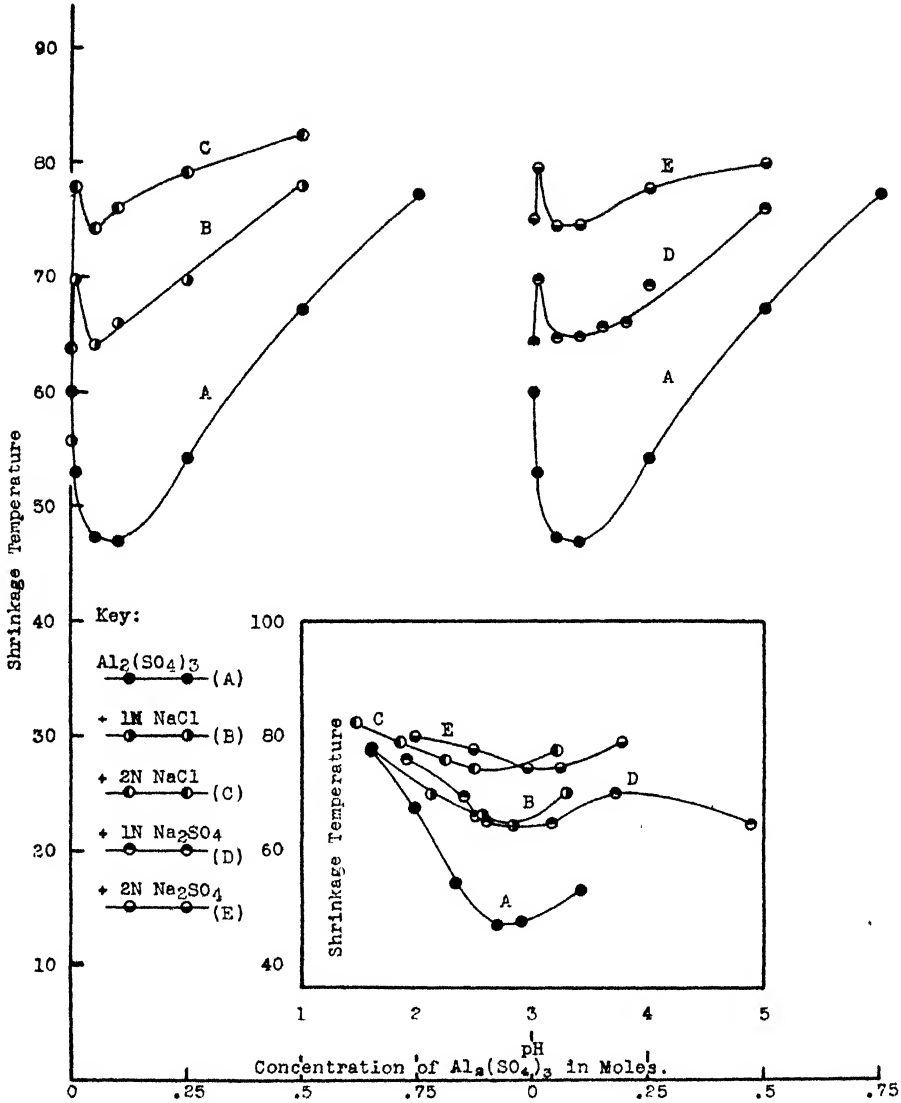


Figure 102. Effect of Aluminum sulfate—sodium chloride pickles upon shrinkage temperature of treated collagen.

causing the protein to become structurally more stable. The addition of sodium chloride causes a shift in the pH value of the minimum stability point from 3.1 to 2.75. It will further be noticed that at the high concentrations of aluminum chloride in the presence of 1 and 2 normal sodium chloride there is a decrease in shrinkage temperature. However, in this region, the pH

value of the solution is less than 1.5, and undoubtedly the sodium chloride is playing an additional role, namely, retarding any fixation of basic aluminum chloride in a manner similar to neutral salts in chrome tanning. Curves B and C of this same figure show the combined role played by aluminum and sodium chlorides. This causes dehydration, thus bringing closer and closer the carbonyl and imino groups of the protein chain and far overbalancing any effect of the structural breakdown of the electrovalent linkages due to the salt and zwitterion discharging effects of the acid aluminum chloride.

Figure 101 gives data for the system ($AlCl_3 - Na_2SO_4$). Curve A of this figure is identical with Curve A of Figure 100. Curves B and C represent data in which the aluminum chloride solutions have been made 1 and 2 normal with respect to sodium sulfate. These curves indicate that sodium sulfate in conjunction with aluminum chloride has a somewhat greater dehydration effect than sodium chloride under the same conditions. At an aluminum chloride concentration of 1 molar and sodium sulfate concentration of 2 molar, the structural stability of the protein closely approximates that resulting from treatment with formaldehyde. Aluminum chloride solutions containing 2 mols sodium sulfate not only retard completely any structural breakdown but actually increase the cohesion forces of the collagen. Both curves indicate a slight minimum structural stability at pH 3; however, using 2 molar sodium chloride solutions, this minimum is barely apparent.

Figure 102 gives shrinkage temperature data for the system [$Al_2(SO_4)_3$], [$Al_2(SO_4)_3$ + NaCl] and [$Na_2(SO_4)$]. Curves A represent the aluminum salt without any additional neutral salt. These curves indicate a more drastic action than that obtaining for aluminum chloride; this action is indicated both in the dilute and concentrated range. Curve A shows the powerful ability of aluminum sulfate at concentrations greater than 0.2 molar to strengthen the cohesion forces of collagen. A comparison of Figures 100 and 102 indicate that there is little difference between the systems ($AlCl_3$ + Na_2SO_4) and [$Al_2(SO_4)_3$ + NaCl] as far as their effect upon the cohesion forces of collagen is concerned.

Figure 103 shows data for such systems as [$Al_2(SO_4)_3$ + Na formate] and [$Al_2(SO_4)_3$ + Na citrate]. Curves B′ and C′ show the tremendous influence [in dilute $Al_2(SO_4)_3$ solutions] of sodium formate upon the strengthening of the cohesion forces, as measured by the shrinkage temperature. This effect is not evident in the more concentrated aluminum sulfate solutions which, however, more or less neutralize the free alkalinity of the sodium formate,

$$Al_2(SO_4)_3 + 2H_2O \longrightarrow 2AlOHSO_4 + H_2SO_4$$
$$HCOONa + H_2O \longrightarrow HCOOH + NaOH$$

lowering the pH value of the solution from approximately 5.0 to 3.0.

Curves B and C show the effect of 1 and 2 normal sodium citrate solutions

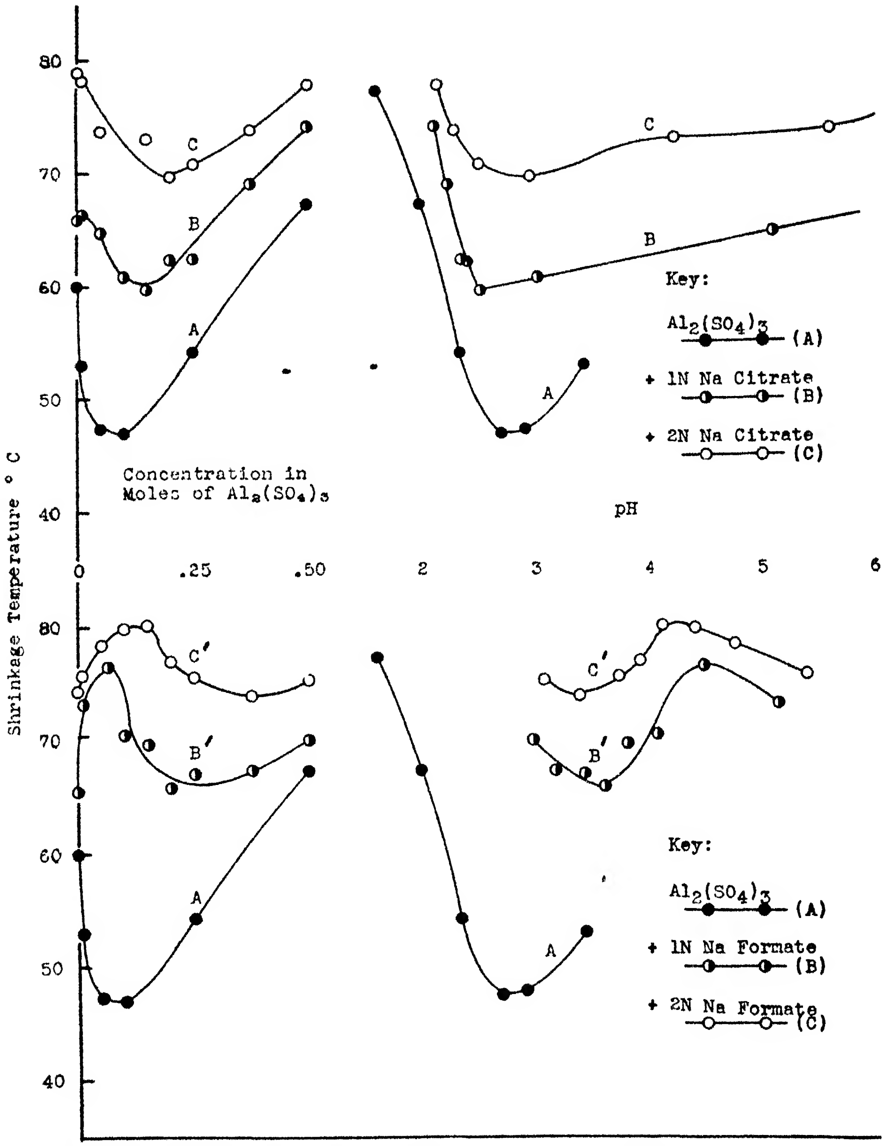


Figure 103. Effect of Aluminum sulfate and organic salt pickles upon shrinkage temperature of treated collagen.

(various concentrations of aluminum sulfate). These curves are similar to those for sodium chloride and sulfate systems.

In a discussion of either aluminum chloride or sulfate systems, it is necessary to take into account an additional factor, the possibility of fixation of basic aluminum complexes. While it is known that the aluminum complex has not the potential stability of the chromium complex, it must be taken into account. If basic aluminum sulfate is fixed by the collagen, then additional strengthening of the cohesion forces will of necessity result.

Pickling for Preservation

All the foregoing material relative to the pickling of hides or skins has been either a discussion of the fundamental principles involved or a discussion relative to the preparation of the skin for mineral tannage. Often times skins are pickled for still another purpose, namely, preservation of the pelt.

Blank[1] in 1932 made a study of the molding of pickled sheep skins. He points out that molds are not exacting in their requirements for growth in that they grow well upon most moist organic material and over a wide range of pH values. Blank divides pickled skins into two main classes: the one, skins which have had only a light pickle, and the other, skins which have been strongly pickled. Skins which ordinarily are "low" pickled are calf and side upper hides. In this case, the skins may have been pickled for only a few hours in a dilute pickle and are expected to be tanned within a few days at most. This type of pickling is in preparation for subsequent chrome tannage. If such skins are held in the "pickled state," mold growth often ensues and may create considerable damage. Blank's report dealt in the main with sheep skins having a "high" pickle since, under specific conditions, they may be held for several months before processing. He divided his investigation into two major parts: one, a general study of molds usually found on pickled skins, and the other a study of certain pickle liquors in relation to their preserving effect. In the first study, he discussed: (a) isolation of the molds; (b) pigmentation; (c) proteolytic activity, and (d) acid and salt tolerance. As a second study, Blank investigated the following pickle solutions: (a) the sulfuric acid-sodium chloride systems; (b) the sulfuric acid-sodium chloride-sodium acetate systems; and (c) the sulfuric acid-sodium chloride-*p*-nitrophenol systems.

Blank was unable to isolate viable organisms from any of the pickle liquors examined, which may possibly be due to the make-up of sulfuric acid and salt added daily. From molded skins, he was able to isolate molds belonging to three major families, together with several types of yeast. The three types of mold found were: (1) one similar to the genus *Hormodendrum* and designated by Blank as H; (2) one belonging to the family *Mucedinaceae*, genus *Penicillium*; and (3) one belonging to the family *Mucedivaceae*, genus *Monilia*.

The last two are designated P and M respectively. Blank believes that these three types of mold growth are fairly representative of those organisms which are common in pickled sheep skins and which cause the most trouble in this regard.

In the matter of pigment formation, tanners are most familiar with the color associated with mold growth; black, green and red seem to predominate. In many cases, these discolorations can be removed during processing; but often they persist even into the finished leather.

In 1930, Thom[21] pointed out that pigment produced by molds may have three distinct locations: the conidia, the mycelium, and the substratum. If the pigment is produced in the conidia, which are the seed-like parts of the mold, it may exist some distance above the surface of the pickled skin and in that case may be easily removed by mechanical means. If on the other hand, the root-like parts of the mycelium produce the pigment, it is much more difficult to remove. If the color does not confine itself to some part of the mold but diffuses into the skin itself, the pigment acts like a dye and is likely to remain throughout subsequent processing.

Blank points out that mold H produces heavily pigmented hyphae; such a discoloration on pickled skin is shown in Plate 10. Pickled sheep skins which have been inoculated with this particular mold on the grain side show growth within a short time on the flesh side. Plates 4-10 show the presence of the mold hyphae on and within the skin.

Blank maintains that mold H is different from *Aspergillus niger*, which has been described at length by Wilson. Wilson found *Aspergillus niger* to be responsible for certain black spots on leather.

Blank points out that mold P produces certain green spores in addition to producing a yellowish red pigment, which readily diffuses into the skin. It is often known as red mold.

A dark-green pigment is produced by mold M, but this pigment remains essentially in the sphores and hyphae and apparently is not as difficult to remove as H and P.

Molds under favorable conditions may secrete a great variety of enzymes. Thom and Church list the following for *Aspergillus niger*: lipase, amylase, inulase, raffinase, gentianase, zymase, invertase, urease and protease. Due to such secretion of enzymes, especially the proteolytic type, the molds may actually digest the skin itself. Mold P rapidly liquefies gelatin, M slowly and H not at all. Blank found that other molds isolated from pickled sheep skin rapidly acted upon gelatin and coagulated blood serum.

The protoplasm of an organism is sensitive to changes in salt and acid concentration. The particular manner in which an organism reacts to such changes in salt concentration is a characteristic of the organism itself. With a slight increase in salt concentration, growth may be stimulated, after which

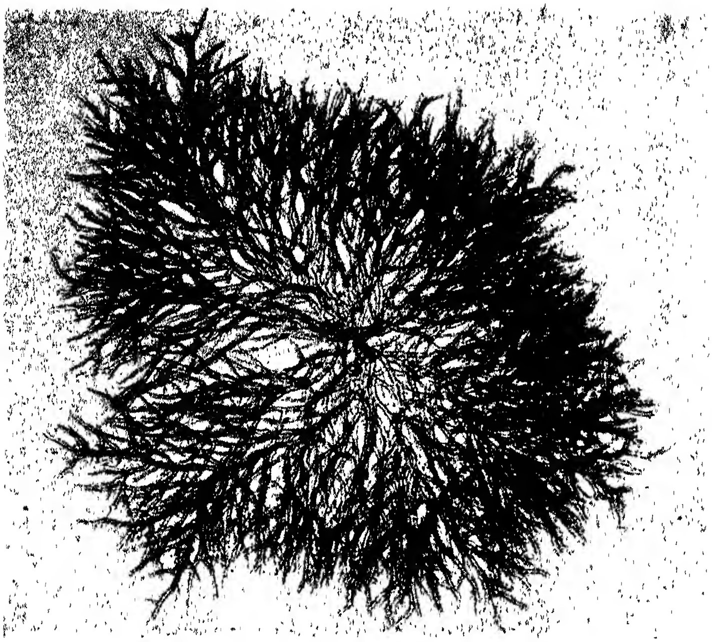


Plate 4.

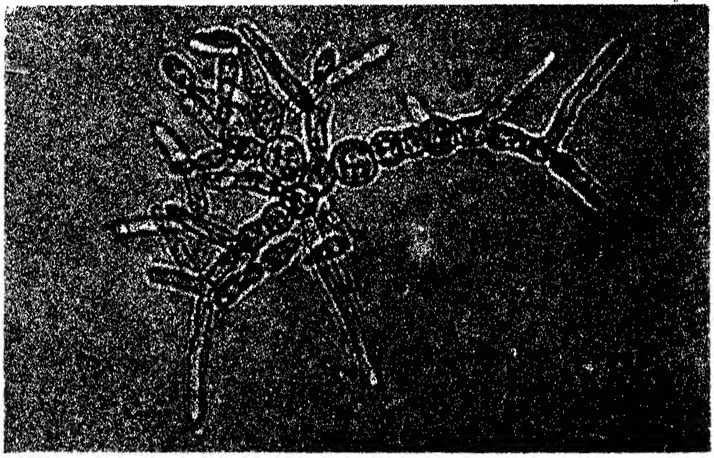


Plate 5.

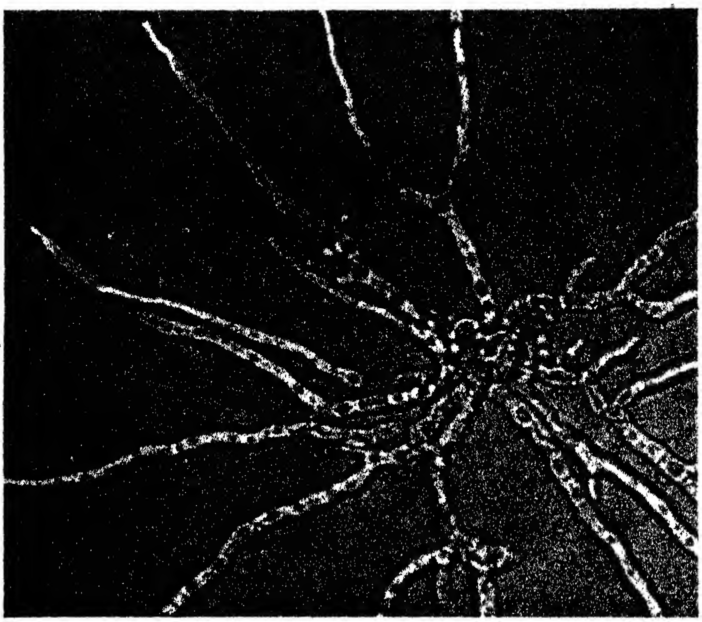


Plate 6.

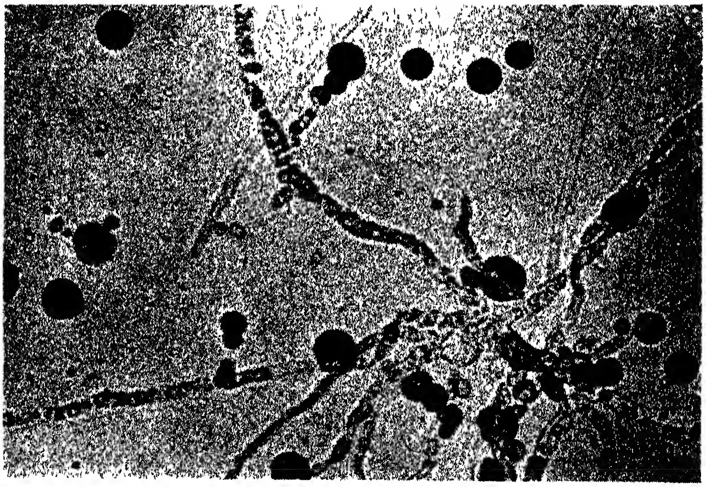


Plate 7.

Plate 8.

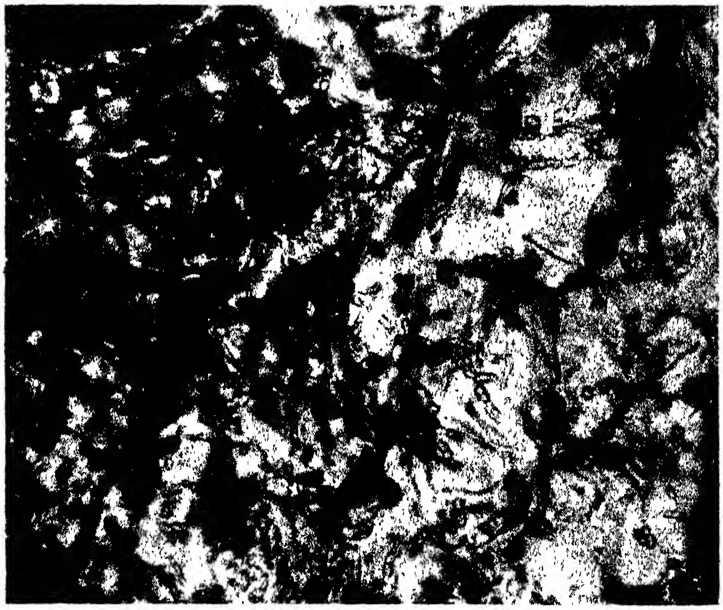


Plate 9.

Plate 10.

further increase may greatly inhibit further growth. The ability to remain viable and to grow in the presence of salt is known as the salt tolerance of an organism.

Doelger[2] found that mold growth is generally inhibited by the presence of more than 12 per cent salt; but some molds have a greater salt tolerance than others. These are given in Table 141.

Table 141. Growth of Molds at 25° in the Presence of Sodium Chloride.

Type of mold	% NaCl permitting growth	% NaCl preventing growth
Alternaria	12	16
A. flavus	16	17
A. niger	17	19
A. repens	12	16
Hormischium	12	16
A. Candidus	20	..

Doelger also investigated the acid tolerance of certain molds. The data are given in Table 142.

Table 142 Maximum Concentrations of Acids which will Permit Growth in Czapek's Broth at 25°.

Type of mold	Sulfuric Normality	Sulfuric pH	Lactic Normality	Lactic pH	Formic Normality	Formic pH
A. niger	0.030	1.87	0.395	1.7	0.018	3.54
A. candidus	0.008	2.65	0.012	3.67	0.003	3 98

Normalities of Acids Necessary to Prevent Growth in Czapek's Broth During 6 Days at 25°.

Type of mold	Acetic acid	Hydrochloric acid
P. camenberti	0.053	0.045
P. brevicaule	0.045	0.038
R. nigricans	0.045	0.028
M. racemosus	0.028	0.044
Hormodendrum	0.028	0.053

Blank studied the salt and acid tolerance of molds isolated from pickled sheep skin and found that at pH 4.3 molds P and M grew well in the presence of 18 pèr cent salt, whereas growth of mold H was inhibited by salt concentrations greater than 9 per cent. With regard to acid tolerance, this investigator found that the various molds tolerate entirely different amounts of acid and that a single mold may tolerate different amounts of different acids. He also noted that acetic acid prevents mold growth at a relatively low hydrogen-ion concentration compared to such acids as sulfuric or hydrochloric. These data are shown in Table 143. Both Doelger and Blank found that acetic and formic acids appear to be toxic to molds in general.

Blank made a further investigation upon the inhibiting effect of certain pickles. He pickled sheep skins using salt and sulfuric acid and then inocu-

Table 143. Maximum Concentrations of Acids at Which Growth Was Observed in Modified Czapek's Medium at 25°.

Type of mold	Sulfuric Normality	Sulfuric pH	Hydrochloric Normality	Hydrochloric pH	Acetic Normality	Acetic pH
H	0.486*	0.61	0.404	0.46	0.041	4.48
P	0.057	2.05	0.071	1.97	0.041	4.48
M	0.057	2.05	0.071	1.97	..†	...

* Highest concentration tried.
† No growth in lowest concentration tried.

lated them with cultures of the molds. He found a satisfactory pickle to contain 12 per cent sodium chloride and 1.5 per cent sulfuric acid. He found that only mold H would grow in a pickle liquor of this type, and that addition to the above pickle of 1 per cent sodium acetate made it much more efficient in preventing mold growth. He also found that the addition of .025 per cent *p*-nitrophenol aided materially in this respect.

Pleass[9] in 1935 made a study of the pickling of sheep skin. She found that the presence of acetic, formic, salicylic or benzoic acid in the sulfuric acid-salt pickles prevents mold growth, and that, although acetic acid and salt did not give a satisfactory pickle liquor, 2 per cent formic acid and 10 per cent salt appeared to be efficient in all respects.

Analysis of Pickle Solutions and Pickled Skin

Since most pickle solutions contain merely sulfuric acid and sodium chloride, their analysis is comparatively simple. In the control of "paddle pickles" it is only necessary to control the salt content with a hydrometer, as the specific gravity of a particular liquor is governed mainly by its salt content, the acid having but little effect. The acidity is usually controlled by titration of a given aliquot of the equilibrium liquor, such an amount of acid being added as will give a standard titration value for any given aliquot taken as a sample.

The analysis of the pickled skin is decidedly more difficult. If the original pickle solution was made up of sulfuric acid and sodium chloride, the liquor contained H^+, Na^+, HSO_4^-, $SO_4^=$ and Cl^- ions. During pickling, the skin proteins combine with some of these ions. Experimental data have shown that the H^+ ions are fixed in relation to the equilibrium pH value of the particular pickle, and that $SO_4^=$ or HSO_4^- ions are bound in greater proportion than Cl^- ions. Therefore, the pickled skin contains H^+, Cl^-, HSO_4^- and $SO_4^=$ ions, some bound to the skin proteins, and others merely being dissolved in the free pickle liquor mechanically held within the skin structure.

If a complete analysis of the pickled skin is desired, the following procedure should be followed:

(1) The sample is divided in half; one-half is allowed to air-dry, and the other is pressed twice at 5-10,000 pounds per square inch and then allowed to air-dry. Both samples are then ground to a powder in a Wiley mill. The samples are then ready for analysis.

(2) Hydrogen ions. One gram of the ground material is weighed into a 250-ml Erlenmeyer flask and 15 ml distilled water is added. The sample is allowed to "wet up" for one hour. To the wetted sample is added a solution containing 2 grams potassium iodide, 0.5 gram potassium iodate, and 25 ml 0.1*N* sodium thiosulfate. The reaction is allowed to go to completion over a 2-hour period and is then back-titrated, either with 0.1*N* iodine solution or 0.1*N* hydrochloric acid in the presence of starch indicator. The ml of standard solution used for back-titration substracted from the 25 ml of 0.1*N* sodium thiosulfate originally used will give the ml of 0.1*N* acid in the pickle sample taken. This can be calculated to grams H_2SO_4.

(3) Sulfate ions. One gram of the sample is digested with 10 ml HNO_3 by boiling until the volume is about 5 ml; it is then cooled, treated with 10 ml HCl and again evaporated to a 5-ml volume. It is then transferred to a 600-ml beaker and neutralized with NH_4OH, using litmus as an indicator. After diluting to about 300 ml with water and adding 5 ml concentrated HCl, the sample is boiled and treated with 10 ml of 10 per cent $BaCl_2$. The precipitated $BaSO_4$ is filtered through a Gooch crucible, washed with warm water, and ignited. Calculation is to $SO_4^{=}$ ion or H_2SO_4.

(4) Chloride ions. One gram of the material is placed in a 125-ml Erlenmeyer flask, to which is added 10 ml concentrated HNO_3 and 25 ml 0.1*N* $AgNO_3$. The mixture is boiled gently until all protein is digested. A little water may be added from time to time so as to have a final volume of about 15 ml always present. After digestion, the solution is cooled, diluted, and the excess $AgNO_3$ titrated with 0.1*N* KCNS in the presence of ferric ammonium sulfate as an indicator. The amount of 0.1*N* $AgNO_3$ used in the precipitation of Cl^- can then be calculated.

(5) Hide substance or protein can be determined by the usual Kjeldahl-Gunning method as given in the official American Leather Chemists Association methods.

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Chapter 12

Aldehyde Tanning

In Volume II of the second edition of this monograph, Wilson discussed fully the data relating to the protein-formaldehyde reaction as it had been developed up to 1928. Since 1928, however, numerous researches have been made and published regarding the mechanism of this reaction. This reaction is of great importance not only in the manufacture of formaldehyde-tanned leather but in any theoretical studies and interpretation of all tannages.

Sorensen[26] in 1907 found that in the titration of amino acids, if formaldehyde was added, a sharp end point resulted in the presence of phenolphthalein. Sorensen believed that water, formaldehyde, amino acid and alkali were in equilibrium and that the reaction might be represented by the equation:

$$HOOC—R—NH_2 + CH_2O \longrightarrow HOOC—R—N{=}CH_2 + H_2O$$

The Sorensen "formol" titration used in protein chemistry is based upon this reaction. Harris[11] preferred to take another viewpoint, namely, that the system is dependent on a compound of an amino acid and formaldehyde and has a dissociation constant different from that of the original amino acid. Harris used the zwitterion concept of Bjerrum[1] and postulated the reaction of the amino acid and formaldehyde as follows:

$$^-OOC—R—NH_3^+ + CH_2O \longrightarrow {}^-OOC—R—NH_2^+\cdot CH_2O + H^+$$

The work of Harris and Birch and Harris gave experimental support to the zwitterion concept of Bjerrum. The titration of glycine in the presence of increasing concentration of formaldehyde is shown in Figure 104. This shows definitely that the addition of formaldehyde in no way affects the titration of glycine in the acid zone, but does markedly affect it in the alkaline region. Adams and Bjerrum had advanced a similar hypothesis in 1916 and 1923. Harris explained the reaction as repression of the acidic ions upon acid titration and of basic ions upon alkaline titration.

Bergmann[3] found that glycine ethyl ester combined with three molecules of formaldehyde, and was further able to isolate a triformal derivative of glycine. He found that this compound changed to the monoformol derivative in alkaline solution. The preparation of the triformal compound of Bergmann led to the widespread belief that in the protein-formaldehyde reaction more than one molecule of formaldehyde combined with each basic group. Investi-

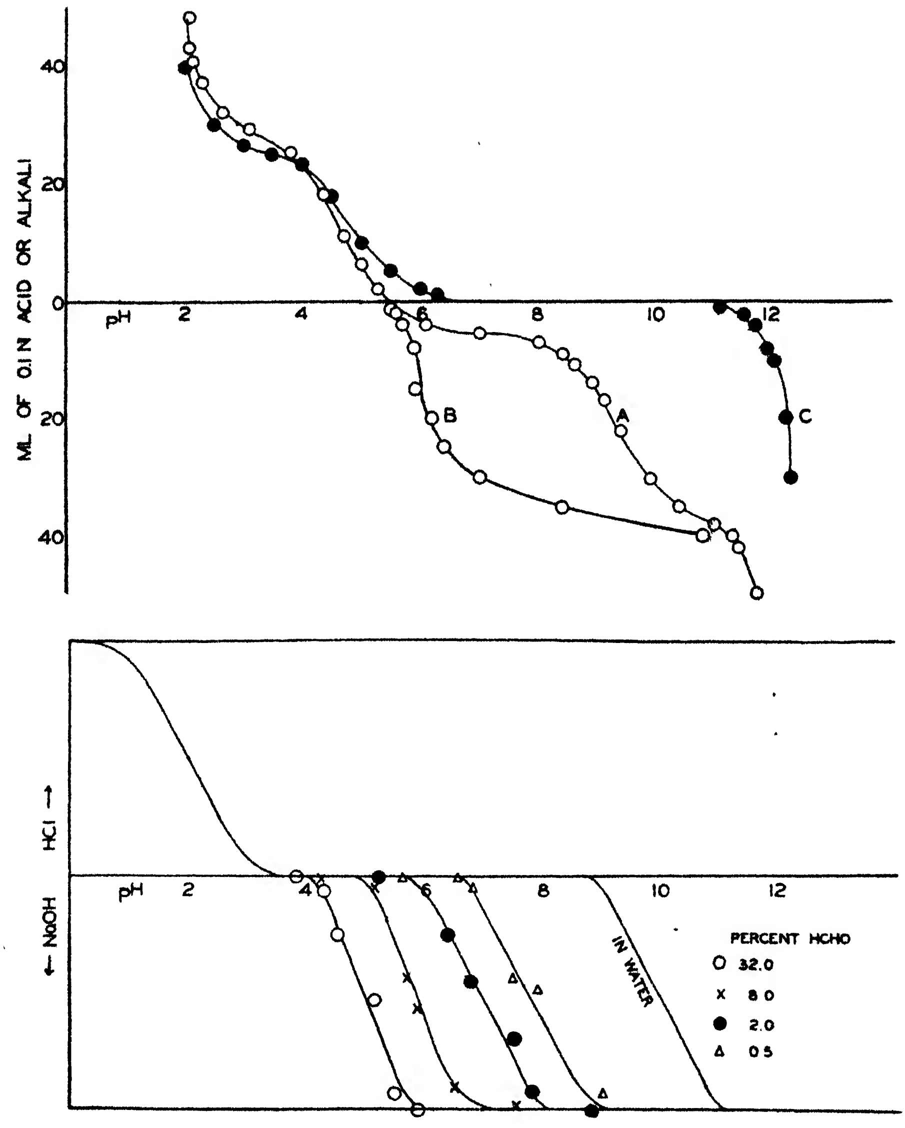


Figure 104

gations based on equilibrium data and equilibrium constants led Tomiyama[33] to the conclusion that the mono-amino acids react with only one molecule of formaldehyde in the pH range 8.0 to 10.0. Tomiyama concluded that only the anionic form of the amino acid, $^{-}OOC{-}R{-}NH_2$, reacted with formaldehyde. He considered the reaction in terms of the electronic theory and

pictured the formaldehyde as a dipolar molecule, $^+CH_2O^-$. Then, since the amino or imino group of the anionic form of the amino acid has two unshared electrons, the two components react to give

$$\begin{array}{c} \mathrm{H} \quad\;\; \mathrm{H} \\ \mathrm{R} : \ddot{\underset{\cdot\cdot}{\mathrm{N}}} : + \ddot{\underset{\cdot\cdot}{\mathrm{C}}} : \ddot{\underset{\cdot\cdot}{\mathrm{O}}} : \\ \mathrm{H} \quad\;\; \mathrm{H} \end{array} \longrightarrow \begin{array}{c} \mathrm{H} \;\; \mathrm{H} \\ \mathrm{R} : \ddot{\underset{\cdot\cdot}{\mathrm{N}}} : \ddot{\underset{\cdot\cdot}{\mathrm{C}}} : \ddot{\underset{\cdot\cdot}{\mathrm{O}}} : \\ \mathrm{H} \;\; \mathrm{H} \end{array}$$

Some investigators suggest, however, that if an associated compound is formed, it might make little difference whether the anionic or the zwitterion form of the amino acid were used, since the proton of the $—NH_3^+$ group is released when the $—NH_3^+$ group reacts with the aldehyde, and this proton is in turn accepted by the anions of the system

$$^-OOC—R—NH_2 + CH_2O \longrightarrow {}^-OOC—R—NH_2 \cdot CH_2O \qquad \text{(I)}$$
$$^-OOC—R—NH_3^+ + CH_2O \longrightarrow {}^-OOC—R—NH_2 \cdot CH_2O + H^+ \qquad \text{(II)}$$

We have but little experimental evidence, however, to substantiate equation II and the present authors are inclined to believe that equation I represents the true facts. A similar mechanism was suggested by Wilson,[37] who also suggested more than a simple co-ordination of the electron-deficient carbon atom of the formaldehyde with the nitrogen atom of the amino group, and postulated long chains of polymerized formaldehyde molecules, $—R—NH_2 \cdot CH_2O \cdot CH_2O \cdot CH_2O$.

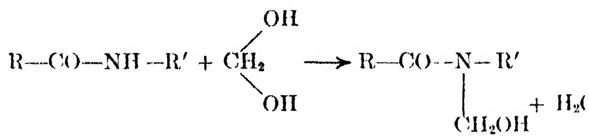
Levy and Silberman[17] criticized the work of Tomiyama and maintained that amino acids may combine with either one or two molecules of formaldehyde. Balson and Lawson[2] pointed out that Tomiyama's investigations were made in relatively dilute formaldehyde solution and that therefore under those conditions it was not surprising that only one molecule of aldehyde reacted. The data indicated that up to three mols of formaldehyde may react. They suggested that the number of formaldehyde molecules which may react depends upon the number of H atoms attached to the N atom, and therefore have proposed the following reactions:

$$NH + CH_2O \longrightarrow N \cdot CH_2OH$$
$$—NH_2 + CH_2O \longrightarrow —NH \cdot CH_2OH + CH_2O \longrightarrow$$
$$—N \begin{matrix} \diagup CH_2OH \\ \diagdown CH_2OH \end{matrix} + CH_2O \longrightarrow —N \begin{matrix} \diagup CH_2O \diagdown \\ \diagdown CH_2O \diagup \end{matrix} CH_2$$

The formation of a triformal derivative is in line with ideas expressed by Stiasny,[28] who believed that formaldehyde first reacted with the basic groups of gelatin, changing these to neutral ones. Stiasny suggested that intermediate triformal derivatives were formed, which then reverted to the monoformal one:

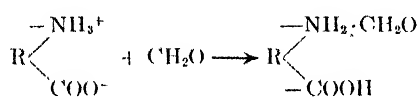
$$R—NH_2 + 3CH_2O \longrightarrow R—N \begin{matrix} \diagup CH_2O \diagdown \\ \diagdown CH_2O \diagup \end{matrix} CH_2 \longrightarrow R—N{=}CH_2 + 2CH_2O$$

After this first reaction occurred, Stiasny believed a second and slower reaction took place at the numerous peptide linkages. He postulated the formation of methylol groups at the imino groups:



Gerngross[10] claimed to have proved that formaldehyde does not react with the imidazole group of histidine; but here again other data dispute this claim. Stiasny has pointed out that the collagen-formaldehyde reaction influences not only the acid and base fixation but also affects the fixation of tanning substances and dyes.

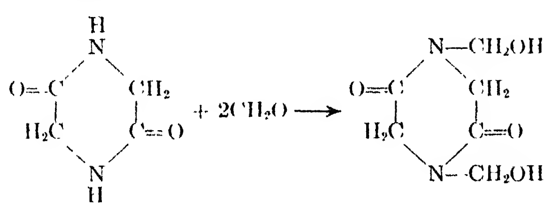
Reiner and Marton[23] suggested the following reaction between protein and formaldehyde:



the aldehyde being held to the freed amino group through secondary valence. Einhour[9] showed that acid amides combine with formaldehyde as follows:

$$R{-}CONH_2 + CH_2O \longrightarrow R{-}CONH \cdot CH_2OH$$

Cherbuliez and Fier,[7] and later Bergmann,[3] found that diketopiperazines react with formaldehyde, taking up two mols of the aldehyde:



Wadsworth and Pangborn[35] have concluded that there may be three stages in the protein-formaldehyde reaction: (1) the formation of a loosely associated compound; (2) the transformation of this into a labile chemical compound; and (3) further transformation into a stable compound.

Studies of the protein-formaldehyde reaction up until 1939 were seriously handicapped by the lack of a positive method of determining formaldehyde fixed by the protein. Gerngross and Gorges[10] used as an index of the amount of tannage the resistance of the formaldehyde-tanned leather to hydrolysis by hot water, as measured by the amount of soluble nitrogen obtained under standardized conditions. The experimental data of Gerngross and Gorges has been fully covered in Volume II of the Second Edition of this monograph.

In 1936, Theis and Schaffer[29] applied to this same problem the measurement of the increase in the shrinkage temperature of skin tanned with formaldehyde compared with untanned skin, when treated with water of increasing temperature. This method, while important, gave no indication of the amounts of aldehyde actually fixed by the protein. In 1938, Highberger and Retzsch[13] developed a method for the determination of bound formaldehyde which has proved both practical and helpful in subsequent experimentation by many investigators. This method is given in full below.

Highberger and Retzsch Method

"The sample of leather to be analyzed should be reduced, by grinding in a Wiley mill or other means, to a fairly fine state of subdivision, similar to that of coarse hide powder. A quantity, amounting to 1 to 2 grams, of the sample thus prepared is weighed into a 500-ml Kjeldahl flask. Ordinarily 2 grams is the most convenient amount to use, but occasions may be encountered where it may be desirable to increase or decrease the amount taken. It is unnecessary to record the sample weight beyond the nearest milligram.

"*Apparatus:* The apparatus used is the simple distillation outfit with vertical condenser shown in Figure 105. A 500-ml Kjeldahl flask, containing the sample and hydrolysing acid, and heated by a Bunsen burner, is connected through a Kjeldahl distilling trap and bent glass tubing to a 250-mm bulb-type Allihn condenser used in the vertical position. The distillation trap is carried in the Kjeldahl flask in a No. 6 rubber stopper, and all other connections are made with rubber tubing, care being taken to see that the glass ends adjoin each other. The lower end of the condenser is connected to a delivery tube which dips beneath the surface of the liquid in a 300-ml Erlenmeyer flask.

"*Distillation:* Sufficient sodium bisulfite solution for the amount of formaldehyde expected is placed in a 300-ml Erlenmeyer flask, which is then placed in position on the apparatus, as shown in Figure 105. A little distilled water may be added to ensure a volume sufficient to prevent the admittance of air when the solution is sucked partially into the condenser. The sodium bisulfite solution is made up to contain 12 grams per liter, and 10 ml of this are ordinarily ample to handle the formaldehyde obtained from the weights of sample specified. As mentioned in the discussion of the titration, a large excess of bisulfite is necessary to ensure complete binding of the formaldehyde. The following schedule will give an idea of the amounts of bisulfite solution necessary to use:

For amounts of formaldehyde up to 0.04 gram use 10 ml bisulfite.
For amounts of formaldehyde up to 0.08 gram use 25 ml bisulfite.
For amounts of formaldehyde up to 0.17 gram use 50 ml bisulfite.

"These figures are based on our own experience of the maximum amounts of formaldehyde that may be safely handled by the quantities of bisulfite specified. In any case where the amount of formaldehyde determined is equal to the maximum for the bisulfite used, it is advisable to repeat the determination using either the same sample weight and double the quantity of bisulfite, or one-half the sample weight and the same quantity of bisulfite.

"The bisulfite solution should be freshly made up at frequent intervals, and should be protected from the air as much as possible. While the addition of many organic substances, such as alcohol or glycerin, exerts a marked stabilizing action, the use of these in the solution before distillation must be avoided. On long standing, and particularly in the aeration which occurs during distillation, some bisulfite is bound by these substances due to oxidation, with the production of a positive error in the subsequent Clausen titration.

"When the receiving flask with the bisulfite solution is in place, 100 ml of approximately $2N$ sulfuric acid are added to the Kjeldahl flask containing the sample, which is immediately connected to the apparatus, and distillation started. The distillation flask is heated with an ordinary Bunsen burner, and the flame is regulated to produce an even boiling, and not too rapid distillation. Trouble with foaming is seldom encountered if care is taken not to heat too rapidly at the start. The flask may be rotated gently to bring down particles of the sample which adhere to the upper walls of the flask, and care must be taken to avoid scorching any part of the sample.

"After a few minutes in most cases the sample will be completely dissolved, except

for any insoluble pigments or other acid resistant material which may be present. The distillation is then continued steadily until only about 10 ml remain in the flask. This usually requires from forty-five minutes to one hour. It is necessary to distill over practically the whole of the liquid in order to obtain a quantitative recovery of the formaldehyde; on the other hand, the distillation must not be continued so far as to produce dryness, or a syrup which will scorch. The distillation is properly stopped when definite white fumes are observed to be starting to form above the residue of liquid in the distilling flask, and before they have become copious enough to be seen in the trap.

"At this point the apparatus is disconnected at the top of the condenser, the flame is removed from under the Kjeldahl flask, and the inside of the condenser is washed down into the receiving flask, using several portions of distilled water. The delivery tube is then removed and washed off into the receiving flask, which is then stoppered and allowed to stand for about 15 minutes.

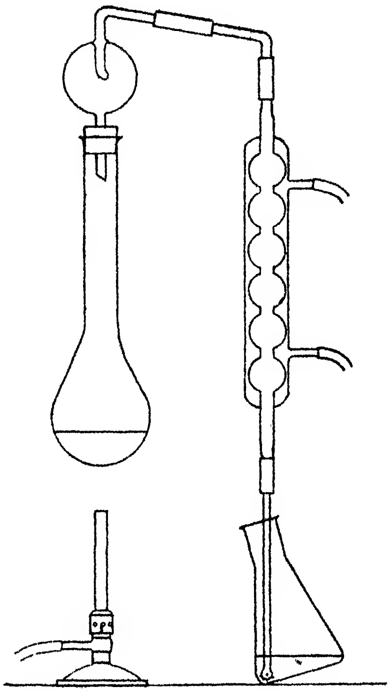


Figure 105

"*Titration:* At the end of the above period 3 to 5 ml of 1 per cent starch indicator are added to the solution, and $0.1N$ iodine is run in until the first blue starch end-point is reached. Care must be taken to adjust this end-point so that it represents an excess of not more than 1 drop of iodine. The quantity of iodine used in this first titration does not enter into the calculation of results.

"When the first end-point is reached, from 10 to 15 ml of 95 per cent ethyl alcohol are added to the solution and well mixed. A quantity of 5 per cent sodium carbonate solution, equal in volume to the volume of 1.2 per cent bisulfite solution originally taken, is then added from a graduated cylinder, and the final titration with $0.1N$ iodine is started as soon as the blue color of the first endpoint is discharged, which should be immediately if there is any formaldehyde present. The titration should be conducted as rapidly as possible, without allowing a large excess of iodine to be present in the solution at any time. If the alkalinity has been correctly adjusted, it will be found that the iodine will be consumed

as rapidly as it can be added, during the greater part of the titration. As the end-point is approached, however, the rate of iodine consumption slows up, and when this occurs 2 or 3 ml more of the carbonate solution may be added. If, in spite of increasing the alkalinity, the rate of iodine consumption is still slow, it is an indication that the end-point is close. It is necessary to add the iodine drop by drop at this stage, swirling the solution in the flask for a few seconds until the blue color fades. Not more than a few drops are required in this manner to reach the sure end-point, which should be perfectly stable even after the addition of a further small quantity (2 or 3 ml) of carbonate solution. Phenolphthalein paper should show a definite red at the end of the titration.

"It must be emphasized that the true end-point of the titration is the definite, rather deep blue color characteristic of iodine-starch titrations. For some reason which is not clear, we have occasionally obtained solutions, usually in the analysis of commercial leathers, in which a faint bluish-pink coloration persisted throughout the second titration. This does not seem to interfere, however, and even under these conditions the sure end-point is readily observed with an accuracy of 1 or 2 drops.

"In the calculation of the results, each ml of $0.1N$ iodine used in the second titration is equivalent to 0.0015 gram formaldehyde (CH_2O)."

While the authors believe the method developed by Highberger and Retzsch is to be preferred, a modified Romijn method used by Bowes and Pleass[5] is given as outlined by those writers.

Bowes and Pleass Method

"The protein was removed from the experimental solution and thoroughly washed on a filter pump with a solution at the same pH as that of the experimental solution after use. The formaldehyde in the protein, immediately after washing, was estimated and in another series of experiments the protein was dried for 6 days at 70 per cent relative humidity before the estimation of the formaldehyde was carried out. In both series the protein was acidified with 40 cc N sulfuric acid and the formaldehyde was driven off by steam distillation through a condenser into a water-cooled flask. It was found that in order to recover all the formaldehyde it was necessary to collect 500 cc of distillate. After mixing well the first 500 cc of distillate, 100 cc was placed in a stoppered flask and mixed with 25 cc N sodium hydroxide and 20 cc $0.5N$ iodine. After one minute 40 cc N sulfuric acid were added and after a further 5 minutes the excess of iodine was back-titrated with $0.1N$ thiosulfate. Standard $0.1N$ thiosulfate was prepared and the concentration of the iodine solution was checked frequently, following the above technique. The amount of formaldehyde recovered from the protein was calculated from the volume of iodine which had combined with the formaldehyde, 1 cc of $0.2N$ iodine solution being equivalent to 0.003 g of formaldehyde:

$$HCHO + I_2 + H_2O = 2HI + HCOOH$$

"The moisture content of the collagen, hair and silk was determined by drying to constant weight in a vacuum oven at 100° C, and all results have been expressed on the weight of protein after drying."

Mechanism of Protein-Formaldehyde Reaction

A study of the literature to date has shown that considerable divergence of opinion exists regarding the mechanism of the protein-formaldehyde reaction.

In 1938, Dyachenoko and Shelpakova[8] studied the tanning of casein by means of formaldehyde. These workers found that in an alkaline solution of pH value 10.4, casein reacts with formaldehyde to form a methylene compound of the type $R\genfrac{}{}{0pt}{}{\diagup N = CH_2}{\diagdown COOH}$, the solution giving a continued rapid

increase in viscosity up to complete gelatinization. They further found that under neutral or weakly acid conditions, 1 mol of formaldehyde reacted with 2 amino groups, forming a methylene bridge, —NH—CH_2—HN—. It was also found that tanning casein by means of other aldehydes gave aldehyde compounds having specific characteristics and represented by the formulas:

—NH—CH_2—HN— | CH_3	—NH—CH_2—HN— | CH | CH_2
acetaldehyde-casein	*acrolein-casein*

Dyachenoko and Shelpakova found that the bridging in casein-aldehyde compounds was a reversible process, being altered by prolonged boiling. Boiling appears to destroy the methylene bridge, splitting off aldehyde quantitatively. In 1939, Holland[15a] suggested that it is desirable to postulate the formation of cross linkages to explain the properties of formaldehyde tannage. He seemed to think that at pH values of 2.0 and 4.0 most of the formaldehyde is attached to the amide groups and is not in the forms of methylene bridges.

The most inclusive researches relative to the protein-formaldehyde reaction conducted since 1936 are those of Highberger[14] and his colleagues and of Theis[30] and his students, in their studies of formaldehyde-tanned leather. This work will be discussed in detail under appropriate headings.

The Effect of Hydrogen-ion Concentration and Formaldehyde Concentration

Early studies of formaldehyde tannage had shown that the pH value of the reaction has a profound effect upon the formaldehyde fixation and upon the physical properties of the leather produced. The reader is referred to the researches of Thomas, Kelly and Foster[32] given in some detail in Volume II of the second edition of this monograph. From 1934 to 1942 Highberger *et al.* and Theis *et al.* made a comprehensive investigation of the influence of pH values upon formaldehyde-fixation by skin collagen, silk fibroin, and wool keratin.

In their investigations, Highberger and Retzsch employed a special purified collagen hide powder and a 24-hour reaction period in phosphate-buffered solutions. At the end of the 24-hour period, the collagen-formaldehyde compound was washed over night in a Wilson-Kern apparatus, using 15-19 liters of distilled water. The pH range studied was from 4.0 to 11.5. The data obtained are shown in Tables 144 to 148 and in Figure 106.

Highberger and Retzsch noted that the curves representing the formaldehyde-fixation as a function of pH form a roughly parallel family for the different concentrations of formaldehyde. With regard to these curves, it was noted: (a) all the curves show a minimum fixation in the acid zone;

Table 144. Two Grams Collagen Powder Tanned 24 Hours in 100 ml 0.1M Phosphate Buffer Solutions Containing Formaldehyde.

Final pH	Grams Formaldehyde per 100 ml	Grams Formaldehyde Fixed per Gram Collagen	Millimols Formaldehyde Fixed per Gram Collagen
5.02	0.25	0.0022	0.07
4.96	1.0	0.0047	0.16
4.82	3.0	0.0097	0.32
5.08	5.0	0.0112	0.37
6.14	0.25	0.0048	0.16
6.15	0.5	0.0072	0.24
6.12	3.0	0.0031	0.44
6.14	5.0	0.0152	0.51
6.92	0.25	0.0074	0.25
6.92	0.5	0.0094	0.31
6.97	1.0	0.0124	0.41
6.91	3.0	0.0147	0.49
6.92	5.0	0.0162	0.54
8.08	0.25	0.0113	0.38
8.02	0.5	0.0126	0.42
8.12	1.0	0.0138	0.46
7.90	3.0	0.0173	0.58
7.85	5.0	0.0191	0.64
10.19	0.25	0.0136	0.45
10.12	0.5	0.0191	0.64
10.02	1 0	0.0231	0.77
9.86	3.0	0.0303	1 01
9.92	5.0	0 0369	1.23

Table 145. Two Grams Collagen Powder Tanned 24 Hours in 100 ml 0.1M Phosphate Buffer Containing 0.25 Gram Formaldehyde.

Final pH	Grams Formaldehyde Fixed per Gram Collagen	Millimols Formaldehyde Fixed per Gram Collagen
8.13	0.0111	0.37
8.16	0.0112	0.37
8.21	0.0115	0.38
8.50	0.0120	0.40
8.60	0.0121	0 40
8.91	0.0131	0.44
8.92	0.0135	0.45
9.84	0.0157	0.52

Table 146. Two Grams Collagen Powder Tanned 24 Hours in 100 ml 0.1M Phosphate Buffer Containing 0.5 Gram Formaldehyde

Final pH	Grams Formaldehyde Fixed per Gram Collagen	Millimols Formaldehyde Fixed per Gram Collagen
7.30	0.0104	0.35
7.40	0.0108	0.36
7.70	0.0116	0.39
7.82	0.0121	0.40
7.93	0.0124	0.41
8.16	0.0127	0.42
8.36	0.0130	0.43
8.71	0.0139	0.46
8.82	0.0143	0.48
8.82	0.0154	0.51
9.25	0.0164	0.55
9.66	0.0182	0.61
9.70	0.0181	0.60
9.82	0.0184	0.61

Table 147. Two Grams Collagen Powder Tanned 24 Hours in 100 ml 0.1M Phosphate Buffer Containing 1.0 Gram Formaldehyde.

Final pH	Grams Formaldehyde Fixed per Gram Collagen	Millimols Formaldehyde Fixed per Gram Collagen
3.77	0.0018	0.06
4.05	0.0035	0.12
4.90	0.0058	0.19
5.30	0.0060	0.20
5.73	0.0084	0.28
5.95	0.0076	0.25
6.28	0.0109	0.37
6.36	0.0104	0.35
6.63	0.0102	0.34
6.66	0.0200	0.40
6.85	0.0113	0.38
6.86	0.0123	0.41
7.07	0.0128	0.43
7.11	0.0122	0.41
7.23	0.0127	0.42
7.48	0.0123	0.41
7.54	0.0125	0.42
7.72	0.0127	0.42
7.86	0.0131	0.44
7.94	0.0131	0.44
8.39	0.0138	0.46
8.60	0.0145	0.48
9.66	0.0203	0.68
11.36	0.0279	0.93

Table 148. Two Grams Collagen Powder Tanned 24 Hours in 100 ml 0.1M Phosphate Buffer Containing 3.0 Grams Formaldehyde.

Final pH	Grams Formaldehyde Fixed per Gram Collagen	Millimols Formaldehyde Fixed per Gram Collagen
3.93	0.0068	0.23
4.09	0.0086	0.29
4.38	0.0092	0.31
4.99	0.0104	0.35
5.06	0.0105	0.35
5.30	0.0110	0.37
5.39	0.0115	0.38
5.50	0.0109	0.37
5.54	0.0111	0.37
5.69	0.0122	0.41
5.70	0.0124	0.41
5.88	0.0121	0.40
5.96	0.0134	0.45
6.17	0.0136	0.45
6.40	0.0144	0.48
6.64	0.0153	0.51
7.28	0.0161	0.54
8.66	0.0197	0 66
9.35	0.0263	0.88
9.37	0.0310	1.03

(b) the amount of formaldehyde fixation increases with increase in pH value; (c) there is a marked difference in the shape of the four curves; (d) those curves representing intermediate formaldehyde concentration show well

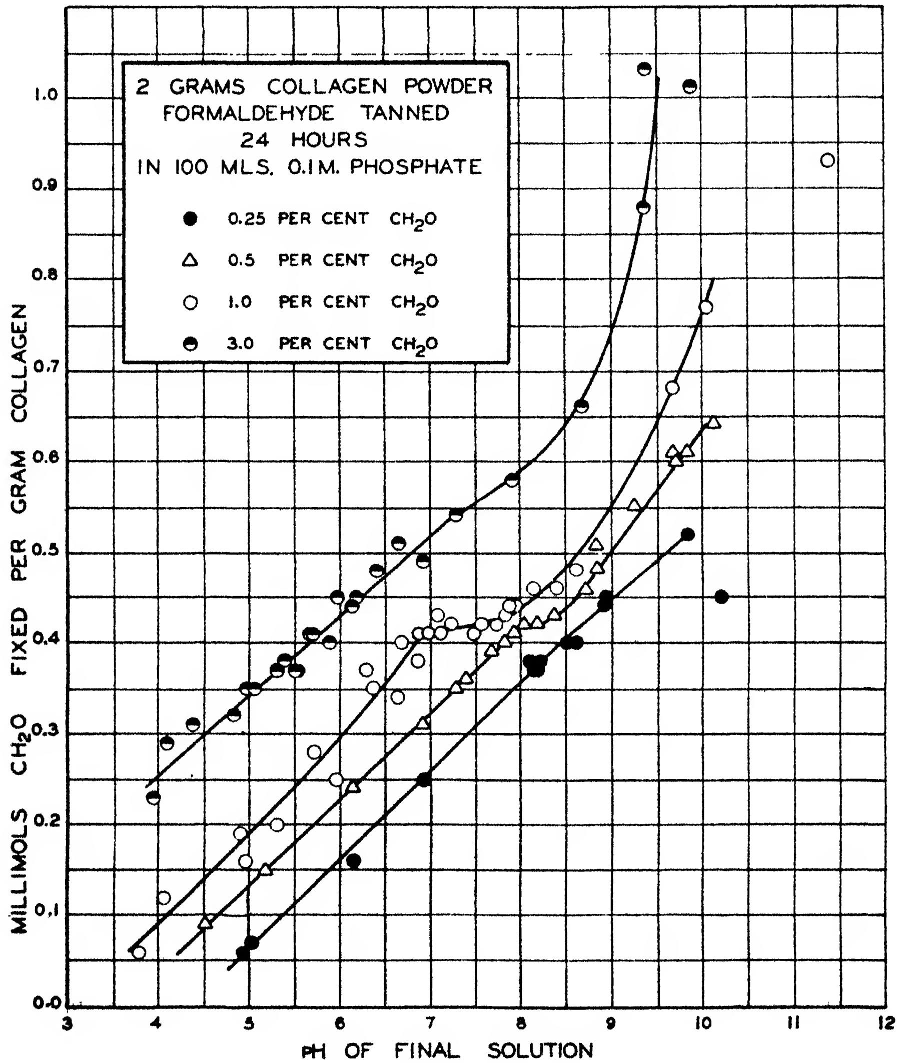


Figure 106

defined breaks at pH values of approximately 7.0 or 8.0; (e) the curve for the lowest concentration is practically a straight line; and (f) the curve for the highest formaldehyde concentration rises very steeply between pH 8.0 and

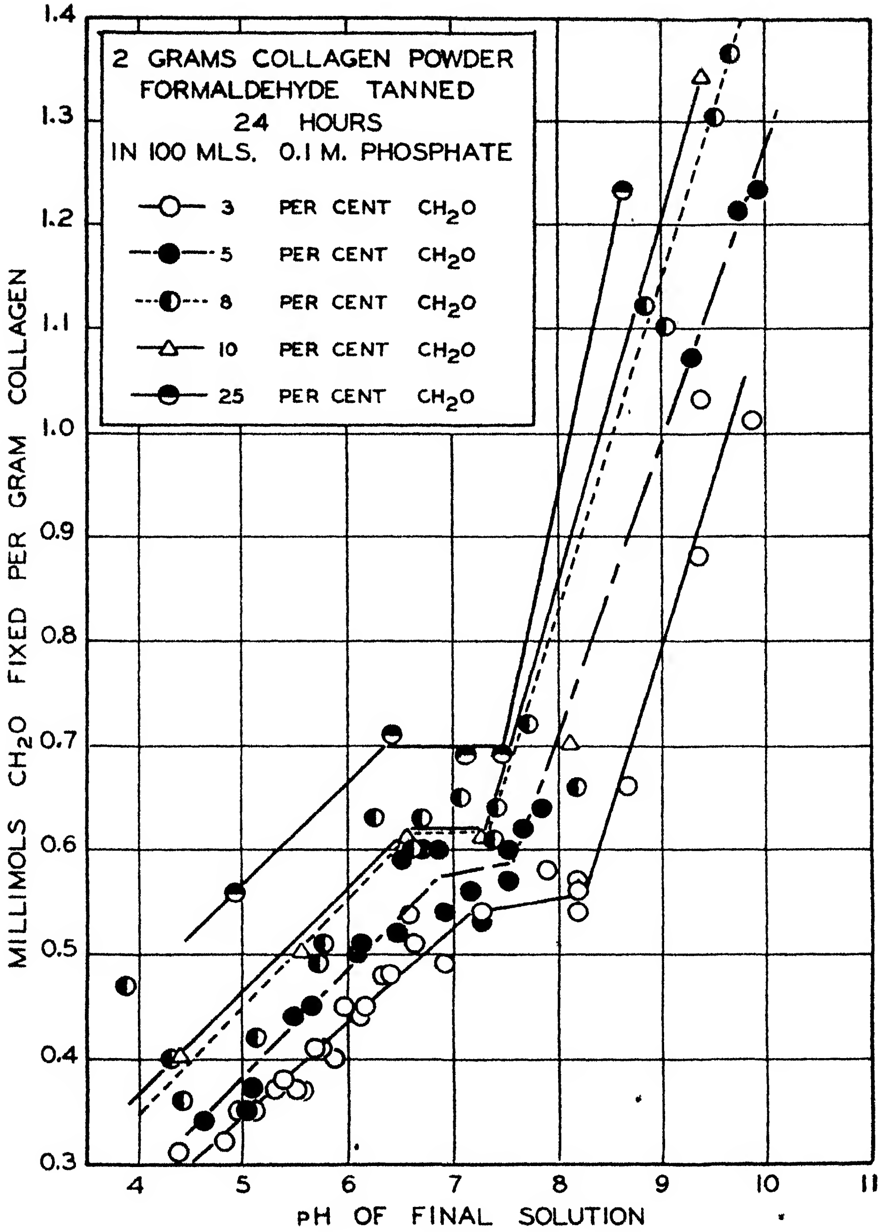

2 GRAMS COLLAGEN POWDER
FORMALDEHYDE TANNED
24 HOURS
IN 100 MLS. 0.1 M. PHOSPHATE
3 PER CENT CH_2O
5 PER CENT CH_2O
8 PER CENT CH_2O
10 PER CENT CH_2O
25 PER CENT CH_2O
MILLIMOLS CH_2O FIXED PER GRAM COLLAGEN
1.4
1.3
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
4
5
6
7
8
9
10
11
pH OF FINAL SOLUTION

Figure 107

10.0. Highberger and Retzsch believe it is significant that the breaks in the formaldehyde-fixation curves occur at approximately 0.4 millimol per gram of collagen, since this value is very close to the amount of lysine known

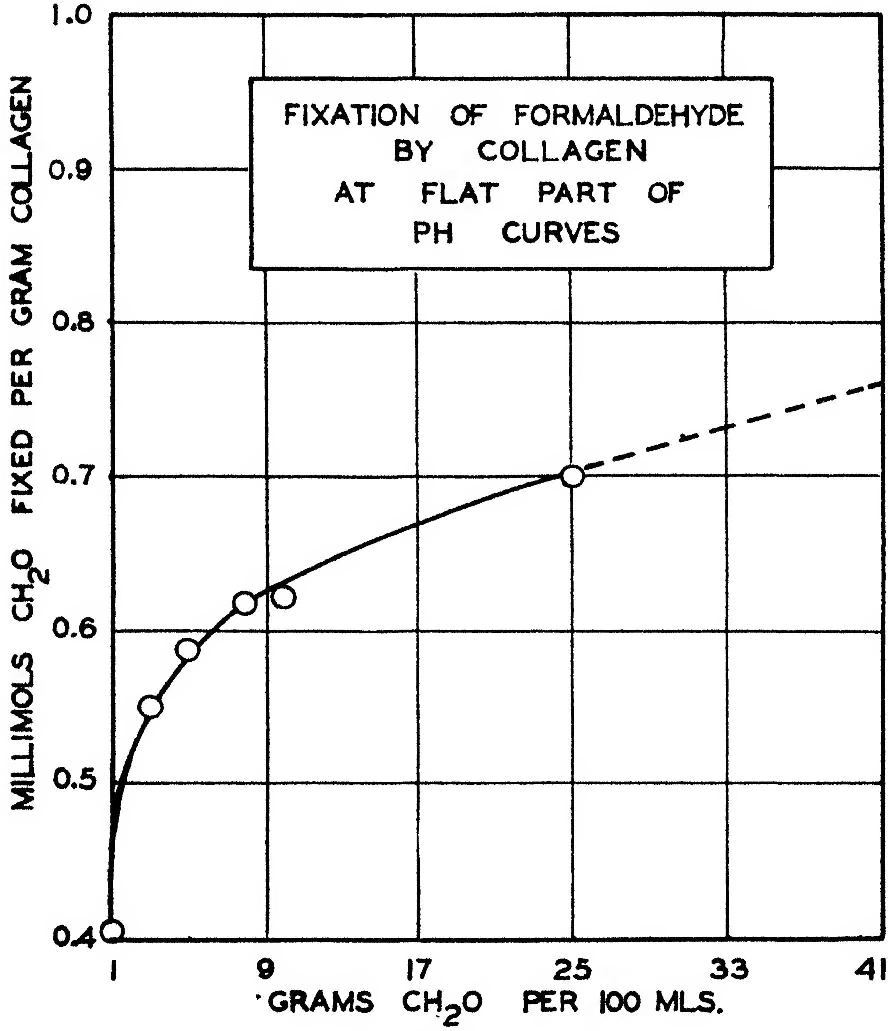


Figure 108

to be present. In the reaction of formaldehyde with collagen, these investigators believe only the undissociated amino groups are involved. They further postulate that the steep rise in the curves at pH values greater than 8.0 is due to combination with the strong basic guanidino groups of arginine.

They make this suggestion even though lysine is not in the completely undissociated state until a pH value of 10 is reached, and arginine not until pH 14.0 is attained.

Highberger and Retzsch point out that with increasing formaldehyde concentration, the formaldehyde may react with the imino groups of the peptide chain, since these particular groups are less basic than the amino or guanidino groups and should be undissociated in the acid zone. This statement, the authors believe, is contradictory to some of their other claims.

In a later work, Highberger and Salcedo point out that the amounts of formaldehyde fixed with collagen increase with increasing concentrations of the formaldehyde solution at all pH values. The curves in this investigation all show definite breaks or flat portions in the isoelectric zone. These investigators point out that the breaks are not all located at the lysine equivalence point, as was the case in their earlier work, but instead occur at higher values for bound aldehyde. They interpret these data as an indication of the freedom of amino and guanidino groups of the collagen to react with more than one molecule of formaldehyde in the more concentrated solutions. They further state that such data preclude any reaction of the imino groups of the polypeptide chain and the formation of methylene bridges. The data are shown in Figures 107 and 108.

Bowes and Pleass[6] made a study similar to that of Highberger and Retzsch, but extended their investigation to include hair and silk. Their work included only one concentration of formaldehyde, namely, a one per cent solution. These investigations showed that silk bound but very little formaldehyde, and that hair and collagen behaved somewhat alike. They came to essentially the same theoretical conclusions as those of Highberger and Retzsch.

Theis and Schaffer[29] in 1936 measured the shrinkage temperature of many varieties of formaldehyde-tanned leathers. They studied the effect of time, temperature, concentration and pH value of formaldehyde tannage. Their results are shown in Figure 109. In a later work (1939) Theis and Priestly[30] augmented their earlier investigations by a study of the increase of shrinkage temperature caused by formaldehyde tannage and by estimation of the bound aldehyde. For this work, bated calf skin was used, at room temperature and for a 24-hour tanning period. After tannage, the formaldehyde-treated skin was washed for several hours with distilled water. For the one per cent formaldehyde solution, the aldehyde-fixation curve was similar to that obtained by Highberger and Retzsch. The data obtained are shown in Table 149 and in Figure 110. The shrinkage temperature measurements indicate little "leathering" in the pH range 1.0 to 2.0; but a drastic increase in this factor occurs at pH values greater than 2.0. From these data Theis and Priestly suggested the concept of earlier workers, namely, the idea of

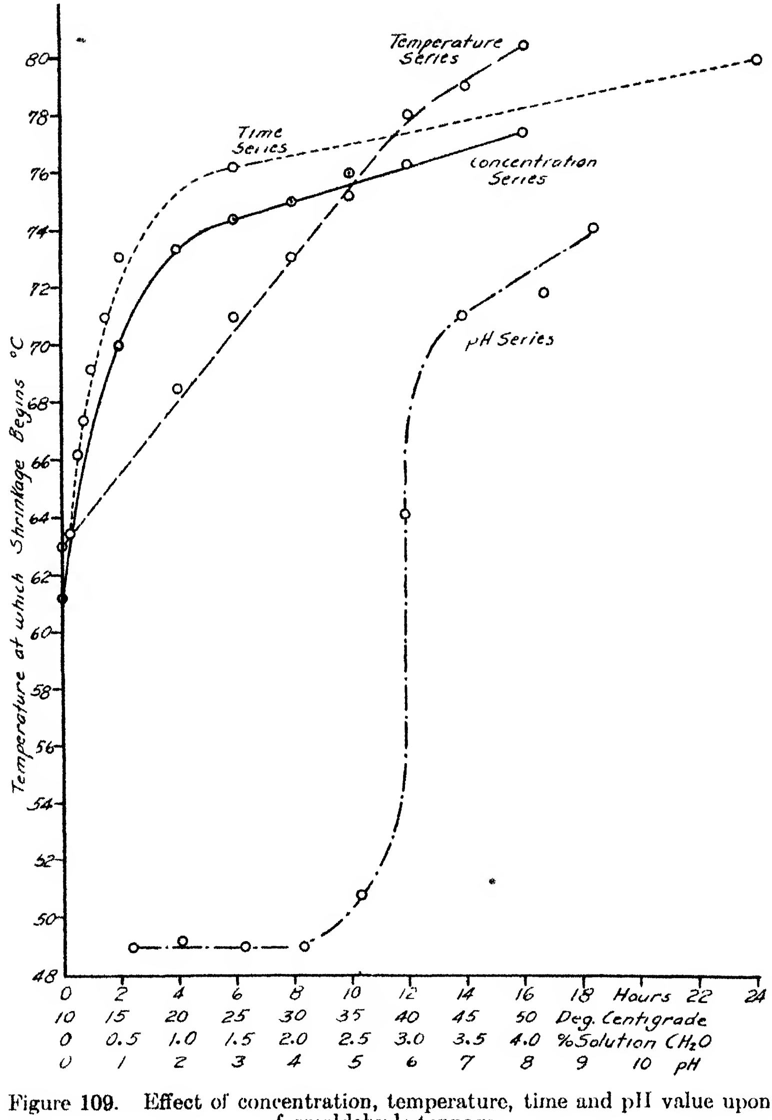


Figure 109. Effect of concentration, temperature, time and pH value upon formaldehyde tannage.

Table 149. Effect of pH upon Formaldehyde-fixation and upon the Resulting Shrinkage Temperature of Leather.

pH	% CH_2O	Shrinkage Temperature	% Shrinkage
1	0.24	45.0	39.0
2	0.17	45.0	53.1
3	0.36	76.0	68.4
4	0.58	80.4	68.4
5	0.39	78.6	67.1
6	0.54	82.0	67.1
7	0.54	82.0	60 6
8	0.92	83.5	60 6
9	1.09	84.0	64.8
10	1.54	84.0	60.9
11	2.59	83.5	46.3
12	3.01	79.5	46.3

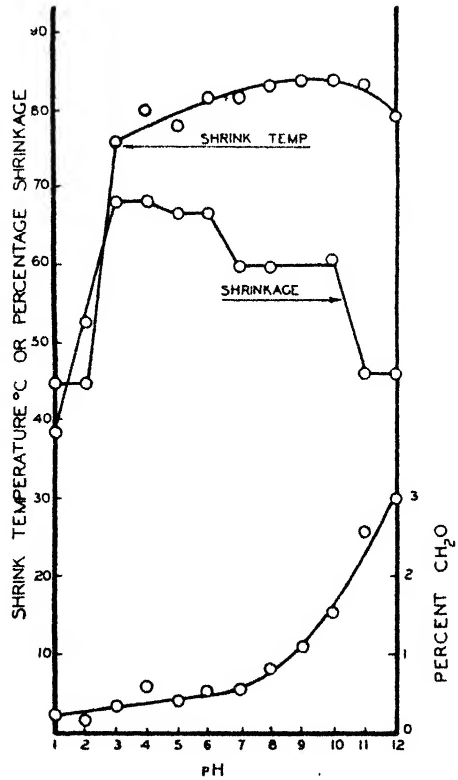


Figure 110. Effect of pH value of formaldehyde upon tannage of bated calf skin.

methylene bridges between adjacent polypeptide chains. They suggested the possibility of formaldehyde reaction between imino groups of adjacent chains in the acid zone $>NH \quad HN< + CH_2O \rightarrow >N{-}CH_2{-}N<$. At pH values greater than that of the isoelectric point, the possibility of methylene bridges between free and undissociated amino groups in juxtaposition was postulated. These claims were based upon the striking increase in resistance to temperature at pH values of 3.0 or more, since the mere exchange of hydrogen atoms for a CH_2 group could hardly account for such great increases in this physical factor.

In this same investigation, Theis and Priestly studied the effect of increasing formaldehyde concentration. Their data are given in Table 150 and in Figure 111.

Table 150.

CH_2O Concentration	pH	% CH_2O*	Mols CH_2O†	Shrinkage Temperature (° C)	% Shrinkage
0.25	2	0.11	1 28	46	47.3
0.50	2	0.17	1.35	47	53.0
1.00	2	0.17	1.58	45	53.1
2.00	2	0.29	3.28	65	64.8
3.00	2	0.41	4.76	73	65.1
4.00	2	0.50	5.16	76	67.2
5.00	2	0.52	5.95	78	67.3
0.25	10	1.66	19.1	81.5	52.9
0.50	10	1.61	18.5	81.5	56.8
1.00	10	1.54	17.7	84.0	60.9
2.00	10	1.95	22.4	83.5	52.8
3.00	10	2.65	30.5	83.0	45.0
4.00	10	2.77	31.8	84.5	47.3
5.00	10	3.21	36.9	82.5	61.0

* Based on hide substance.
† Moles CH_2O per mole collagen.

Examination of these data reveals that at pH 2.0, the increase of shrinkage temperature of the collagen-formaldehyde compound changes drastically with rising concentration of formaldehyde, while at pH 10.0, such increase is very slight. The increase in bound formaldehyde rises strikingly with increase in formaldehyde concentration at all reasonable pH values. It is difficult to imagine the fully dissociated lysine amino groups reacting with formaldehyde at pH 2.0, as suggested by Highberger *et al.* It would seem to the authors that swelling, hydration and reaction with acid amide and imino groups would be the more reasonable explanation.

In order to free the protein-formaldehyde compound of occluded free formaldehyde, Highberger and his colleagues used either distilled water or a very dilute sulfite solution in a continuous washing process. Highberger believed that such a method would remove the free formaldehyde from the protein-formaldehyde compound and not in any way influence the fixed

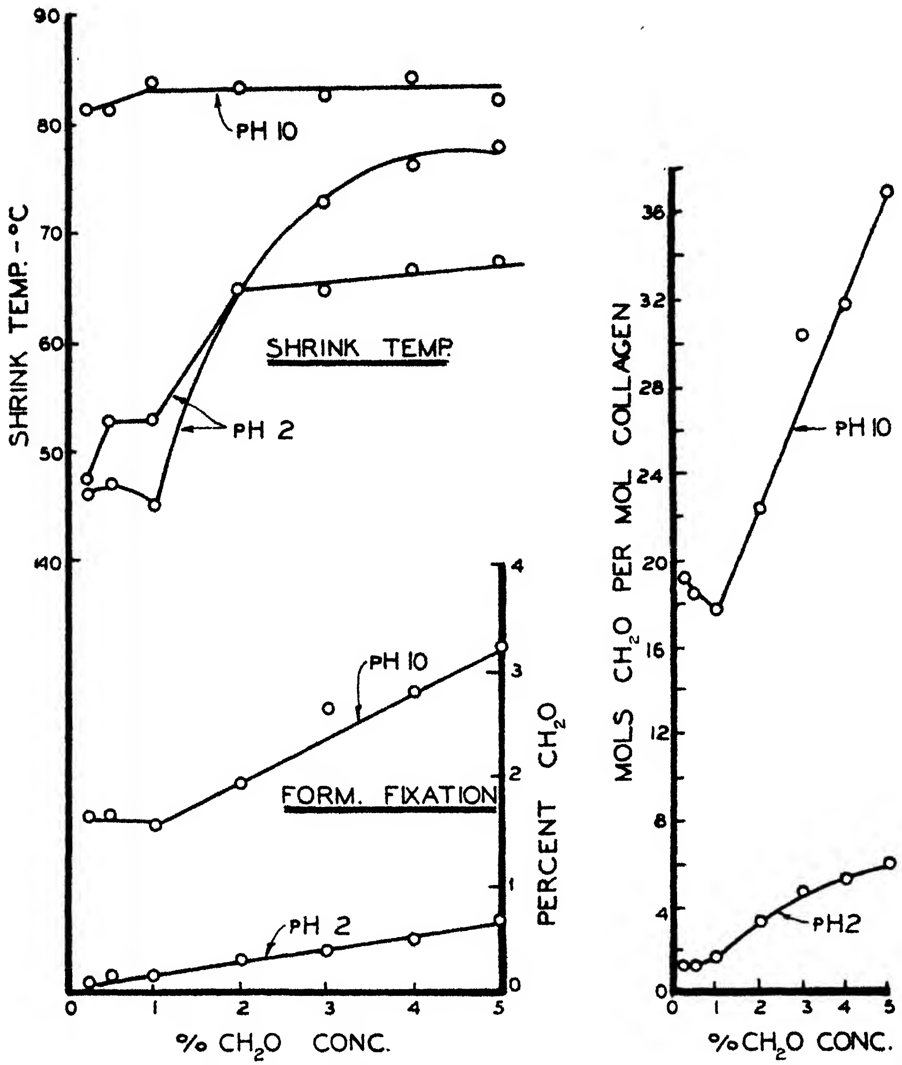


Figure 111. Effect of formaldehyde concentration during tanning upon formaldehyde fixation—upon shrinkage—upon leathering.

formaldehyde. Theis and Ottens found that continuous washing with water removed fixed formaldehyde, even when the pH value of the wash water was maintained the same as that of the residual tan solution. They also found that sulfite washing gave low results. Realizing the extreme importance of this factor in studying formaldehyde tanning, Theis and Lams[30] investigated the high-pressure technique of McLaughlin and Adams,[18] which had been

used very successfully by them in the study of acid- and base-combining capacity of collagen.[19] This technique has been previously described; it involves pressing the collagen-formaldehyde compound several times under pressures of 5-10,000 pounds per square inch. Both McLaughlin and Theis have shown in their published works that this pressure removes essentially all of the free water and free electrolyte. In applying this particular technique to the protein-formaldehyde compound, it was assumed that pressure would remove all the free or unbound formaldehyde.

Using this technique, Theis and Lams reinvestigated the formaldehyde-fixation of collagen, wool-keratin and silk-fibroin over a wide pH range and at several different formaldehyde concentrations. The data obtained for collagen in this study are shown in Table 151 and in Figure 112. They show not only the formaldehyde-fixation but also the acid and base bound by the collagen-formaldehyde compound. Curve A shows the normal acid- and base-binding capacity of native collagen: a maximum acid fixation of 0.87 milliequivalent per gram of protein; a maximum base fixation of 0.38 milliequivalent; an isoionic point of 6.5; a wide plateau in the pH range 7.0 to 9.0 indicative of the back-titration of histidine; and a sharp point of inflection at pH 10.0, beginning the back-titration of the basic groups of lysine. Curve B represents the titration curve of the collagen treated with acidic or basic one-per cent formaldehyde. This curve is identical with A in the pH range 0.8 to 9.0. There is no indication of a shift in the isoionic point of the treated collagen.

In the pH range 9.0 to 11.0, more base is bound than is the case for the untreated collagen, indicative of the reaction between the amino groups of lysine and formaldehyde. The two curves A and B merge at pH 12.0 and approach a maximum base-binding value. Curve C represents the acid and base bound by collagen in the presence of 5 per cent formaldehyde. This curve is also identical with that of A in the pH range 0.8 to 7.0 and definitely shows no shift in the isoionic point. However, due to the large excess of formaldehyde present during the reaction, definitely more base is bound in the pH range 7.0 to 12.0. The binding of base in this region strongly indicates that the basic groups of lysine have been more or less changed by the formaldehyde. At pH 12.0, all the titration curves appear to merge and tend toward a maximum base-binding value. Curve D pictures the fixed formaldehyde in the one-per cent formaldehyde fixed at pH 1.0, thereafter increasing almost as a straight-line function of increasing pH to 0.45 milliequivalent at pH 6.5, or at the isoionic point. Here there is a definite break in the curve, the fixed formaldehyde increasing to 0.5 milliequivalent, remaining constant from pH 7.0 to 9.5. At pH 9.5 the curve shows a decided point of inflection, the formaldehyde fixed increasing sharply up to pH 11.5, when a maximum value, approximately 0.87 milliequivalent aldehyde, is attained. At pH 11.5 there

Table 151. The Acid, Base and Formaldehyde Bound by Collagen.

0.0 % CH_2O	
pH	H^+ or OH^-*
0.9	0.84
1.5	.84
2.2	.79
3.9	.50
5.1	.17
6.2	.04
6.5	.00
6.8	.06
7.4	.09
7.8	.09
8.7	.10
9.7	.13
10.4	.20
11 4	.31
12.2	.40
12.4	.40

0.25 % CH_2O	
pH	CH_2O*
5.0	0.11
5.8	.29
7.0	.35
8.2	45
9.4	.53
10.4	.67
11.0	.74
12.1	.87
12.8	86

0.50 % CH_2O	
pH	CH_2O*
0.9	0.13
1.9	.14
3.1	.17
4.6	.23
5.3	.25
5.7	.28
6.1	.30
6.5	.31
7.5	.40
8.2	.49
8.9	.49
10.4	.74
11.6	.83
12.2	.85
12.7	.87
13.0	.87

1.0 % CH_2O		
pH	H^+ or OH^-*	CH_2O
1.1	0.83	0.15
2.2	.81	.19
3.3	.66	.23
4.1	.44	.28
5.0	.16	.35
5.4	.12	.38
5.9	+.06	.40
6.4	.00	.42
6 9	−.09	.48
7.8	.09	.48
8.8	10	.48
9.3	.18	.48
9.9	.23	.67
10.9	32	.83
11.7	.36	.87
12.4	.38	.93

2.0 % CH_2O	
pH	CH_2O*
5.6	.45
6.6	.65
7.5	.69
8.8	.70
11.8	1.25
12.3	1.30
12.8	.95

3.0 % CH_2O	
pH	CH_2O
5.5	.53
6.4	.65
7.6	.70
8.4	.73
11.8	1.15
12.3	1.40
12.8	1.05

5.0 % CH_2O		
pH	H^+ or OH^-*	CH_2O
.9	.84	.15
2.0	.85	.18
2.9	.69	.43
4.1	.41	.63
5.1	.20	.68
5.4	+.13	.65
6.2	+.02	.74
6.6	−.03	.77
7.1	−.10	.78
7.7	−.19	.94
8.9	−.31	1.17
10.6	−.45	1.33
11.1		1.38
11.4	−.48	1.50
12.2	.54	1.55
12.9	.59	1.25

* All values given in millimols per gram of protein.

is again a break in the curve, indicative of a reaction with the basic groups of arginine.

Figure 113 shows the effect of formaldehyde concentration upon aldehyde fixation by collagen. The formaldehyde concentration varied from 0.25 to 5.0 per cent. Curves A, B, C, and D are identical with those shown in

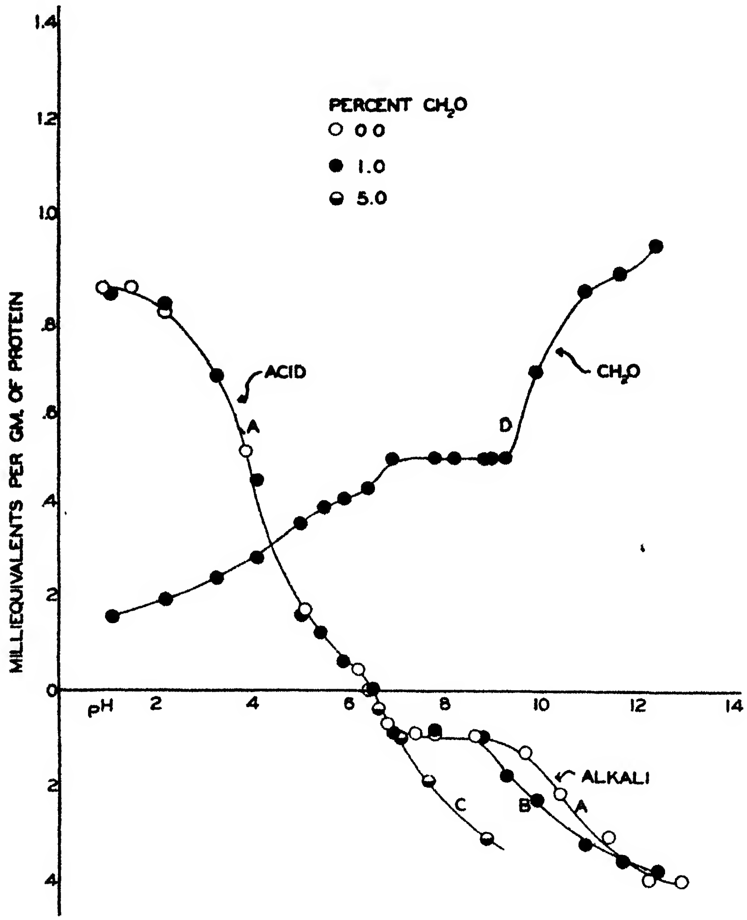


Figure 112

Figure 112. Curve E represents the fixed formaldehyde from the 0.25 per cent formaldehyde solution. This curve shows a marked break at pH 9.5 and only a slight indication of a plateau zone, but shows approximately the same aldehyde-fixation at 12.5 as Curve D. Curve F represents the fixed formaldehyde from the 0.5 per cent solution. This curve shows a slightly

lower aldehyde-fixation in the acid zone as compared with Curve D, a plateau in the pH zone 8 to 9.5, and approximately the same aldehyde fixation as D at pH values greater than 9.5. Curves G, H, and I represent the higher concentrations of formaldehyde. G and H show a definite point of inflection

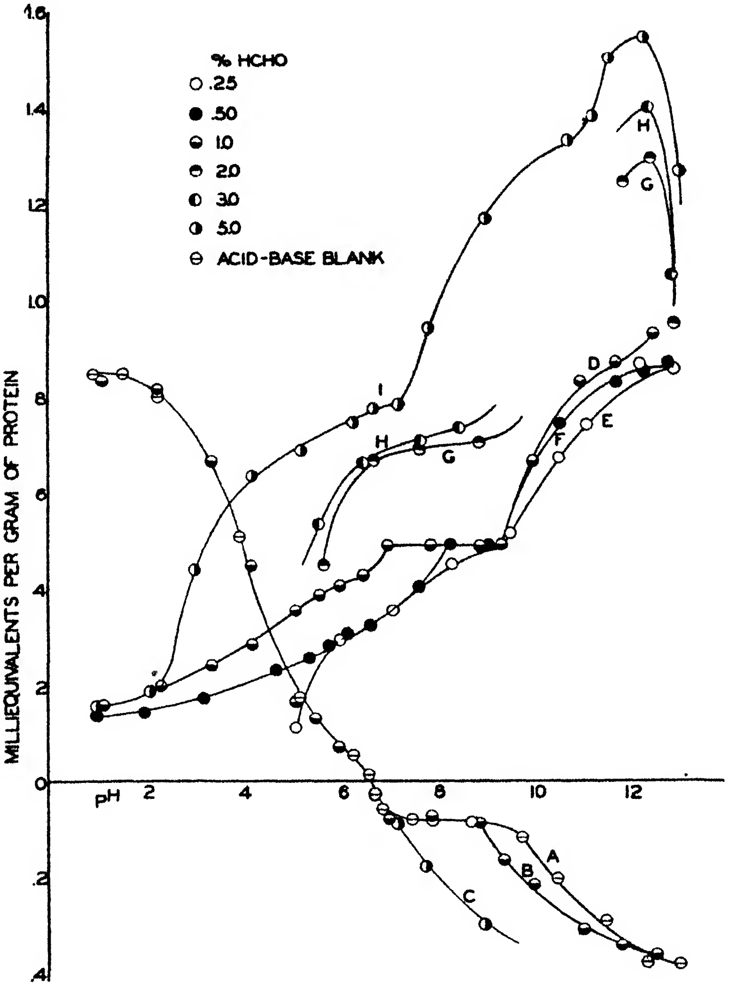


Figure 113

and even strong indications of a plateau zone in the pH range 6.5 to 8.5. These curves show a decided increase in aldehyde fixation at pH 12.0. Curve I shows a definitely greater formaldehyde-fixation in the acid zone with a definite point of inflection at pH 7.0 and a maximum value of 1.6 milliequiv-

alents formaldehyde bound at pH 12.0. Curves G, H, and I all show a certain decrease in aldehyde fixation at pH values greater than 12.0 because the Cannizzaro reaction takes place in this range.

The data secured by Theis and Lams, although they indicate somewhat the same trend as do those given by Highberger, are not in agreement in certain zones, as can be seen from Figure 114, in which the washing method used by Highberger *et al.* and of Bowes and Pleass is compared with the pressing method used by Theis. The plateau zones shown by Theis are much broader than those

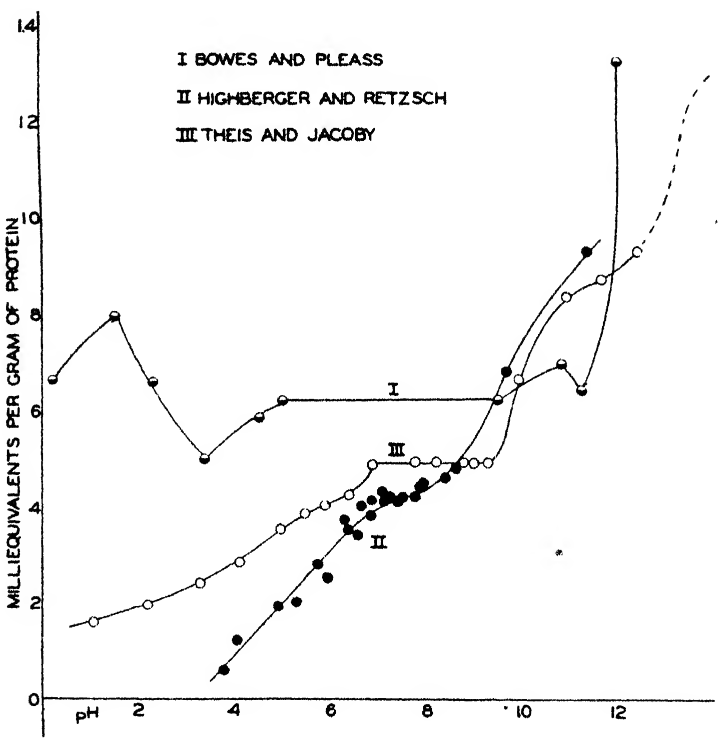


Figure 114

given by Highberger. These zones are of extreme importance, since they represent the trend of a particular reaction. Highberger claims this reaction to be the binding of formaldehyde with the amino groups of lysine, but Theis believes the flat plateau region represents the binding of formaldehyde by histidine (since this plateau corresponds to a similar one in the normal titration curve). This suggestion is at variance with the expressions of other investigators in this field. Theis and Lams further suggest that in moderate concentrations of formaldehyde, lysine is not affected until after the histidine

has reacted, or not until a pH of 9.5 is reached. It is in the pH range of 9.5 to 12.0 that the amino groups of lysine react with the formaldehyde. To explain the trend of their curves, Theis and Lams suggest that in the acid zone and up to pH 6.5, formaldehyde reacts with the weakly basic imino groups in juxtaposition, forming methylene bonds or bridges between polypeptide chains:

$$>NH \quad CH_2O \quad HN< \dashrightarrow\; >N{-}{-}{-}CH_2{-}{-}{-}N< + H_2O$$

At pH 6.5 histidine reacts with the formaldehyde, giving rise to a plateau region in the curve, this zone corresponding to a similar zone in the normal

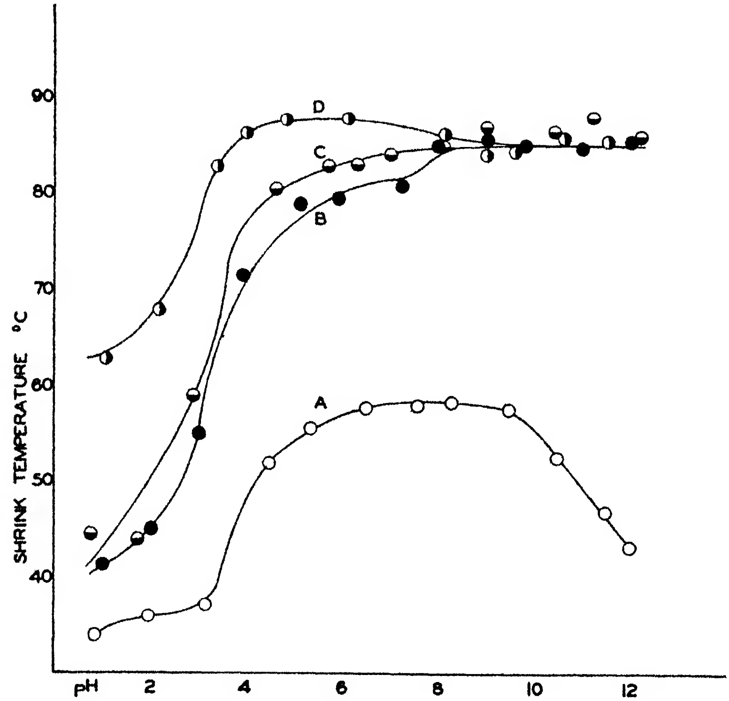


Figure 115

titration curve. As the amino groups of lysine come into play, a definite point of inflection is noted and the fixation of formaldehyde increases sharply. At about pH 12.0, the formaldehyde-fixation curve tends toward a maximum. Theis and Lams suggest that the guanidino groups of arginine play no part in the normal formaldehyde reaction. They point out that only if the reaction were carried out at pH values of 13.0 or higher would these basic groups react with the aldehyde. This is definitely shown by the break in the curves at

pH 11.5. In the ranges of pH 9.0 to 12.0, lysine appears to combine with one molecule of formaldehyde. At higher concentrations of formaldehyde, it is apparent that lysine may combine with 2 moles of formaldehyde. Theis and Lams point out from their data that it seems entirely likely that over the entire pH range, formaldehyde reacts with imino groups of the polypeptide chain. They offer as a proof of this statement the data relative to the shrinkage temperature of formaldehyde-treated collagen. Figure 115 shows these data. This figure demonstrates that even in very low concentrations of formaldehyde (0.5 per cent) increase in shrinkage temperature occurs over the entire pH range. In the acid zone, pH 3.0 to 6.0, the shrinkage temperature increases from 55° to 80°. At pH values greater than 6.0, it increases to 85°. With a 1.0 per cent formaldehyde solution, the shrinkage temperature follows the same trend as that shown for the 0.5 per cent solution; but in the acid zone, pH 3.0 to 7.0, the shrinkage temperature of the collagen-formaldehyde compound is slightly higher. With a 5 per cent formaldehyde solution, a sharp increase in shrinkage temperature takes place in the acid zone, pH 1.0 to 6.0. This is undoubtedly due to a mass action effect, causing a greater number of imino groups to react, resulting in a greater number of methylene bridges. Curves B, C, and D follow the same trend, as shown for the curves representing formaldehyde fixation in Figure 113.

In the considered judgment of the authors, there are two distinct reactions of formaldehyde with collagen in the pH range 1.0 to 11.0; (1) the binding of aldehyde with the free basic imino groups in juxtaposition, which accounts in the main for the increased structural cohesive forces. This particular binding increases with pH value up to the isoionic point, and then remains essentially constant with increasing pH value. (2) The increased fixation of formaldehyde at pH values greater than the isoionic point with the more basic groups of histidine and lysine. In the authors' opinion, this second type of reaction adds materially to the fixed formaldehyde, but little or nothing to the structural network of the collagen-formaldehyde compound. This opinion is borne out remarkably well in the actual manufacture of formaldehyde-tanned leather. Practice has shown that leather produced in the pH range 5.0 to 8.5 is definitely superior to that produced at higher pH values, regardless of the fact that more formaldehyde is fixed at the higher pH values.

The Effect of Arginine Destruction on Formaldehyde Fixation

Sakaguchi[24] found that treating proteins in strongly alkaline solution with sodium hypochlorite destroyed the arginine. Histidine, tyrosine and tryptophane were partly destroyed by this treatment. Highberger and Salcedo used a modification of Sakaguchi's process for making a collagen free of arginine. The process used by these investigators is as follows:

"To 275 grams of collagen powder contained in a large flask surrounded by an ice bath, 2 liters of distilled water were added and the mixture was allowed to stir mechanically for about 15 minutes, after which 700 ml of a sodium hypochlorite solution containing 5 per cent available chlorine were added. The mixture was again allowed to stir for about 15 minutes, after which 2 liters of 1.0N sodium hydroxide were added in portions, the addition requiring about 15 minutes. After some further stirring, the temperature of the mixture had been lowered to about 2°, and the drop-wise addition of 2700 ml more of previously chilled sodium hypochlorite solution was begun. This required 4 hours, during which time the temperature of the mixture was maintained close to 0°, and mechanical stirring was continued as vigorously as possible. After all the hypochlorite solution had been added the mixture was allowed to stir for about 45 minutes more at the same temperature. The material was then removed from the flask and strained through a fine-meshed cloth. After soaking in 0.5N acetic acid for a few minutes, it was restrained and finally allowed to soak in acetic acid of the same strength over night in the ice box. An exhaustive washing with distilled water followed, after which the material was dehydrated in alcohol, and finally reground in the Wiley mill."

Table 152. Two Grams Hypochlorite-treated Collagen Powder Tanned 24 Hours in 100 ml 0.1M Phosphate Buffer Containing 1.0 Gram Formaldehyde.

Final pH	Grams Formaldehyde Fixed per Gram Collagen	Millimols Formaldehyde Fixed per Gram Collagen
3.88	0.0033	0.11
4.77	0.0039	0.13
4.83	0.0041	0.14
5.61	0.0051	0.17
6.02	0.0063	0.21
6.55	0.0079	0.26
7 15	0.0086	0.29
7.50	0.0087	0.29
7.74	0 0093	0.31
8.18	0.0098	0 33
8.41	0.0103	0.34
8.71	0.0110	0.37
8 98	0.0128	0.43
9.50	0.0158	0.53
9.87	0.0162	0.54
10.10	0.0177	0.59
10.33	0.0182	0 61
10.43	0.0180	0.60
10 57	0.0187	0.62
10.83	0.0191	0.64
10.96	0.0205	0.68
11 01	0.0197	0.66
11.02	0.0192	0.64
11 08	0.0201	0.67
11 09	0.0220	0.72

Using the hypochlorite-treated collagen, Highberger and Salcedo studied its formaldehyde-fixation capacity over the pH range 4.0 to 11.0. Their results are shown in Table 152 and in Figure 116. Highberger and Salcedo noted that the general trend of the fixation curve of the hypochlorite-treated collagen was very similar to that for the untreated collagen but that the amounts of formaldehyde combined with the former are lower over the whole range, with the exception of the very acid end of the curve. These investigators claim that the essential point to be noted is that the effect of the hypochlorite treatment on the formaldehyde-fixation capacity of the collagen is greatest

above pH 8.0 and reaches its maximum in the extreme alkaline range, which is the effect to be expected if the additional fixation of formaldehyde in alkaline solution is due to the basic groups of arginine. Highberger and Salcedo then

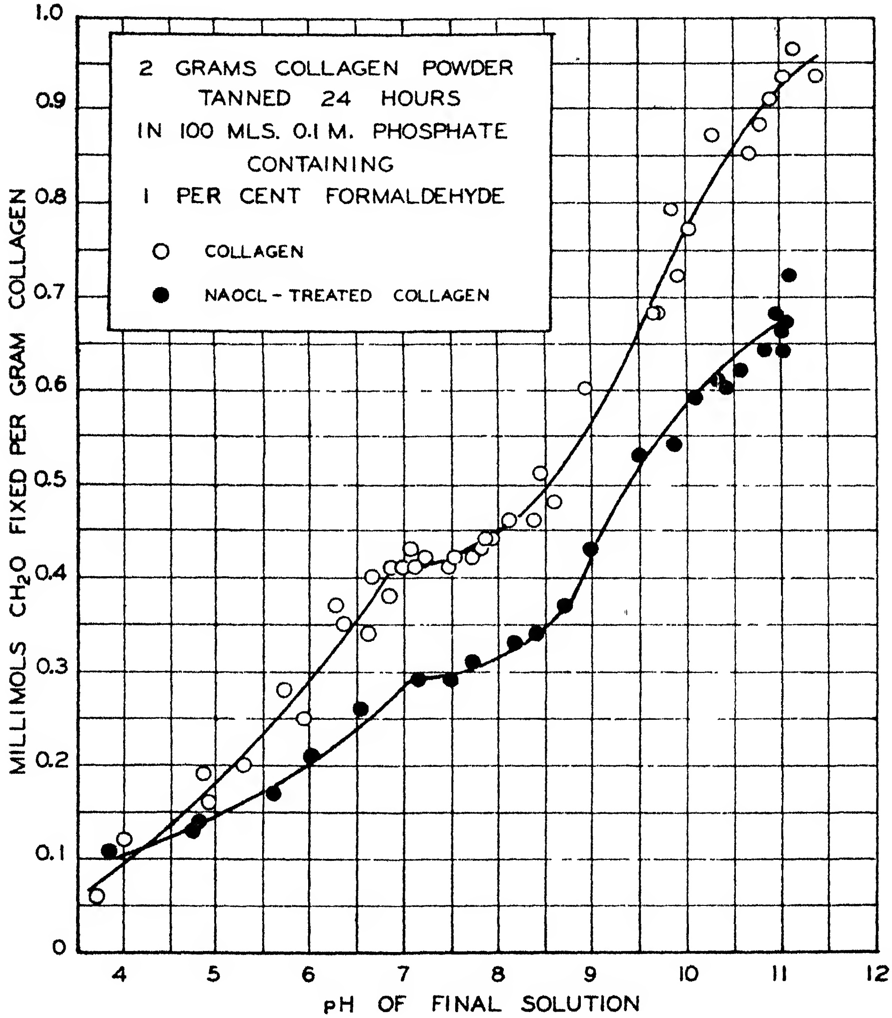


Figure 116

make the claim that their data confirm their original suggestion that the additional formaldehyde fixation by collagen from alkaline solution is due to the combination of the formaldehyde with the guanidino groups of arginine.

The Effect of Deaminization on Formaldehyde Fixation

When amino acids or proteins are treated with nitrous acid, a reaction occurs resulting, it is believed, in a loss of nitrogen from free amino groups. This reaction may be pictured:

$$\text{(I)} \quad \underset{\displaystyle CH_2-COOH}{\overset{\displaystyle NH_2}{|}} + HONO \longrightarrow \underset{\displaystyle CH_2-COOH}{\overset{\displaystyle OH}{|}} + N_2 + H_2O$$

$$\text{(II)} \quad \underset{\displaystyle R\cdot COOH}{\overset{\displaystyle NH_2}{|}} + HONO \longrightarrow \underset{\displaystyle R-COOH}{\overset{\displaystyle OH}{|}} + N_2 + H_2O$$

Investigations over a great many years appear to indicate that in the case of proteins only the ε-amino groups of lysine are affected by treatment with nitrous acid. Van Slyke[38] made use of this reagent in the estimation of lysine nitrogen in his classical protein nitrogen distribution methods. Wiley and Lewis,[36] Vickery and Levenworth[34] and Stendel and Schumann[27] have confirmed the destruction of lysine during nitrous acid treatment. Wiley and Lewis maintain, however, that during such treatment, arginine remains unaffected. Stendel and Schumann take issue with this claim and assert that the arginine residue of the protein is partially destroyed. Both Wiley and Lewis and Stengel and Schumann claim some 50 per cent destruction of histidine during the nitrous acid treatment.

Prideaux and Woods[21] point out that the deaminization must be done in the cold; otherwise, certain side reactions ensue which may result in unaccountable changes. These investigators claim the formation of nitroso groups at ordinary room temperature. The deaminization methods usually employed are those of Thomas and Foster or of Highberger and Retzsch. This method is:

> "Fifty grams of the powdered material were soaked thoroughly in about 500 ml of distilled water, after which 250 ml of a solution containing 50 grams of sodium nitrite were added and the mixture allowed to stir for about an hour. At the end of this time 42.5 grams of glacial acetic acid were added slowly, with stirring. The suspension was stirred continuously for several hours, and finally allowed to stand for 24 hours with frequent stirring. After pouring off the excess solution, the collagen was washed thoroughly in running tap water, and finally in a large number of changes of distilled water. It was then dehydrated in several changes of 95 per cent ethyl alcohol, after which it was air-dried at room temperature."

Meunier and Schweikert[20] in 1935 investigated the properties of deaminized collagen. Their data indicated that deaminization caused a decrease in formaldehyde fixation, that such difference was much greater at pH values less than 7.0 and that in no case was the ability to bind formaldehyde lost entirely. Highberger and Retzsch reinvestigated the formaldehyde-binding capacity of deaminized collagen. For this work, they used a specially prepared collagen material. After deaminization, they studied the formalde-

hyde-binding capacity over a wide pH range. These data are shown in Table 153 and in Figure 117.

These investigators point out that their formaldehyde-fixation curve for the deaminized collagen is very similar to that for the normal collagen, that that amounts of formaldehyde fixed are much lower in all cases, that the slope of the deaminized curve is much less steep below pH 7.0, while above pH 8.0 the slope of the curve is about the same as that for normal collagen. Highberger claims that the low aldehyde-binding capacity of deaminized collagen, at pH values less than 8.0 is due to residual lysine residues. The rather steep rise in the curve at pH values greater than 8.5 is attributed to the reaction of the guanidino groups of arginine with formaldehyde.

Table 153. Fixation of Formaldehyde by Deaminized Collagen.
2.000 grams of deaminized collagen powder tanned 24 hours in 100 ml. 0.1M phosphate buffer containing 1.0 gram formaldehyde.

Final pH	Grams Formaldehyde Fixed per Gram Deaminized Collagen	Millimols Formaldehyde Fixed per Gram Deaminized Collagen
5.16	0.0014	0.05
6.07	0.0029	0.10
6.96	0.0049	0.16
7.27	0.0036	0.12
7.70	0.0049	0.16
8.24	0.0045	0.15
8.50	0.0056	0.19
8.61	0.0054	0.18
8.95	0.0091	0.30
9.56	0.0134	0.45
9.80	0.0147	0.49
10.05	0.0158	0.53

Bowes and Pleass,[6] at about the same time as Highberger and Retzsch, investigated the protein-formaldehyde reaction with both normal and deaminized collagen and hair. In the main, they followed the same procedure as used by Highberger and Retzsch. The data of these workers show little formaldehyde fixation for either collagen or hair at pH values less than 10.0. They conclude that it is the amino groups of lysine and arginine which are responsible for the binding of the aldehyde. They further conclude that in the case of the keratin proteins, other groups than lysine are affected by the nitrous acid deaminization and that there is some evidence of cystine sulfur being oxidized to sulfate.

Deaminization with nitrous acid must certainly destroy a few of the electrovalent salt linkages existing between adjacent polypeptide chains. Such changes would then evidence themselves in the break-down of internal cohesional forces. The breakdown of salt linkages, $-NH_3^+ \ {}^-OOC-$, should be especially marked in the so-called pH stability zone or around the isoionic point. A study of Figures 118 and 118A indicates that such a suggestion is borne out by the facts. Curve A represents the shrinkage temperature of deamin-

ized collagen over the pH range 1.0 to 12.0 and shows definitely that in the region of real break-down of the salt linkages, pH 6.0 to 8.0, there is a sharp decrease in the internal cohesive forces of the collagen. A comparison of this

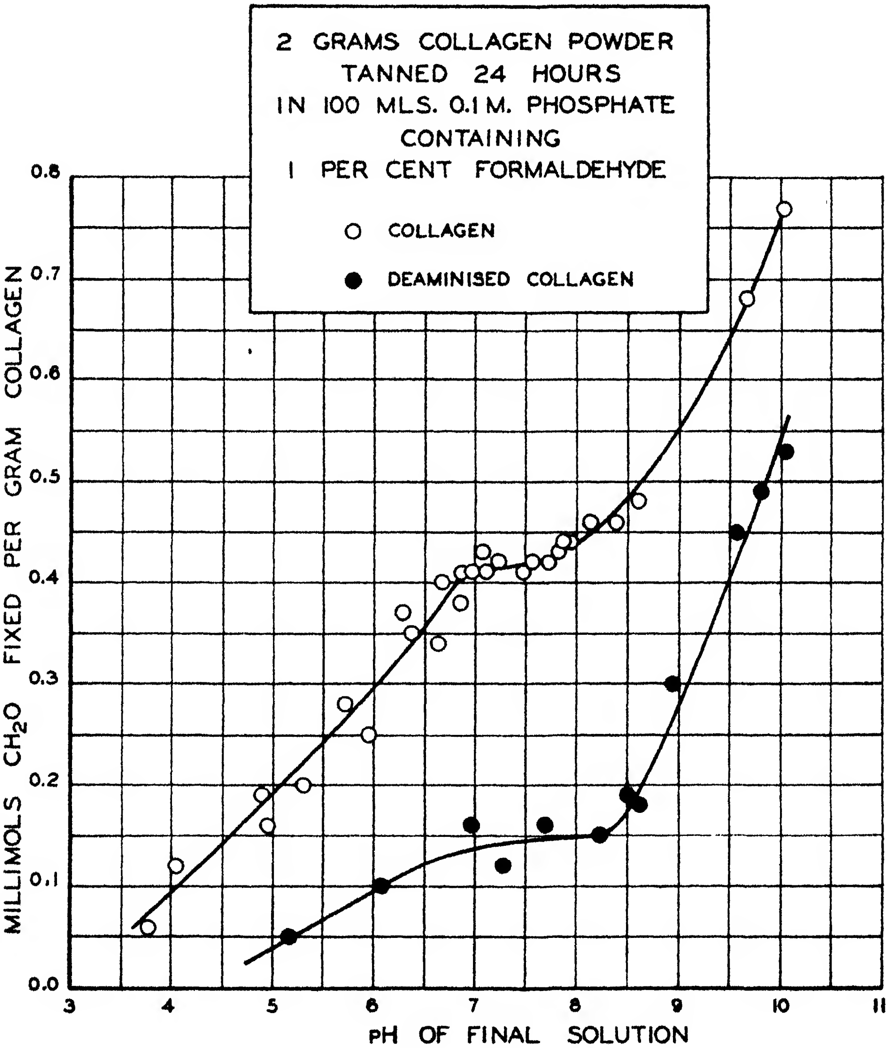


Figure 117

curve with that shown in Figure 115 for normal collagen shows that in the acid zone pH 3.0 to 5.0, the shrinkage temperature of the deaminized collagen is just a little greater than that for normal collagen; this is undoubtedly accounted

for by the decreased swelling of the deaminized collagen. In the alkaline range, pH 6.0 to 12.0, the shrinkage temperature shows a positive decrease in the pH range which would normally be attributed to histidine and lysine. Curve B of Figure 118 shows the shrinkage temperature of formaldehyde-treated deaminized collagen. The data indicate that in spite of formaldehyde fixa-

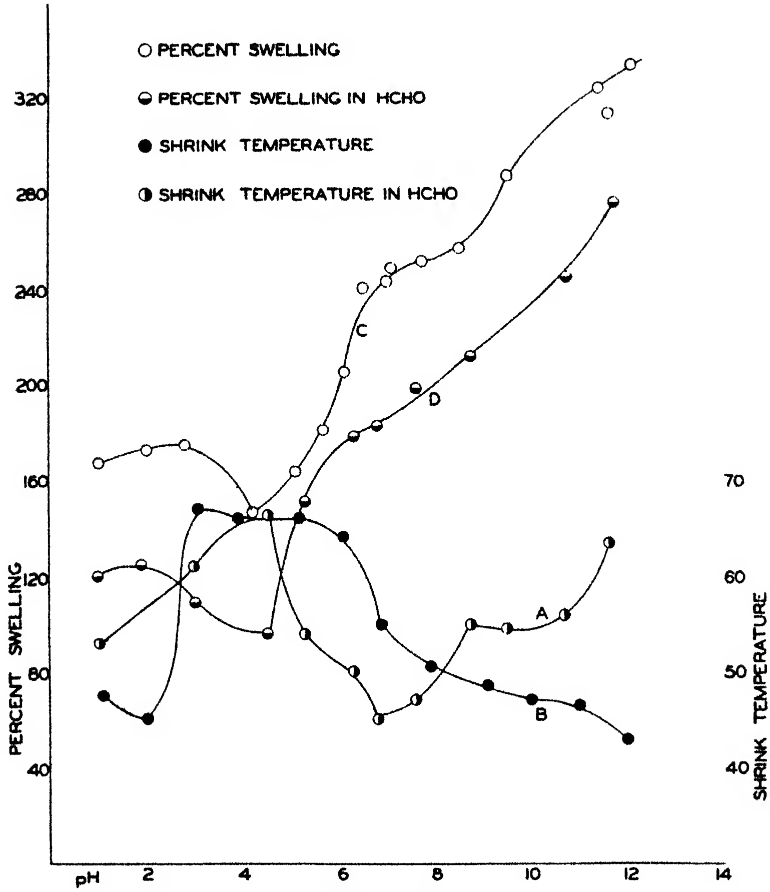


Figure 118

tion, no strengthening of the cohesive forces occurs in the pH range 3.0 to 5.0. At pH values 5.0 to 8.0 an actual decrease in shrinkage temperature occurs. At pH values greater than 8.0 some slight increase in shrinkage temperature results, but hardly greater than that actually obtained for normal collagen; and in this pH zone Highberger shows from 0.2 to 0.5 millimol fixed formaldehyde.

Theis has indicated several times that swelling and shrinkage temperature must of necessity be correlated. This is very well evidenced by a study of curves C and D, representing the swelling of deaminized collagen both with and without formaldehyde treatment. These particular curves show positively that the great swelling taking place in the alkaline region is responsible for the weakening in internal structure of deaminized collagen. Data taken

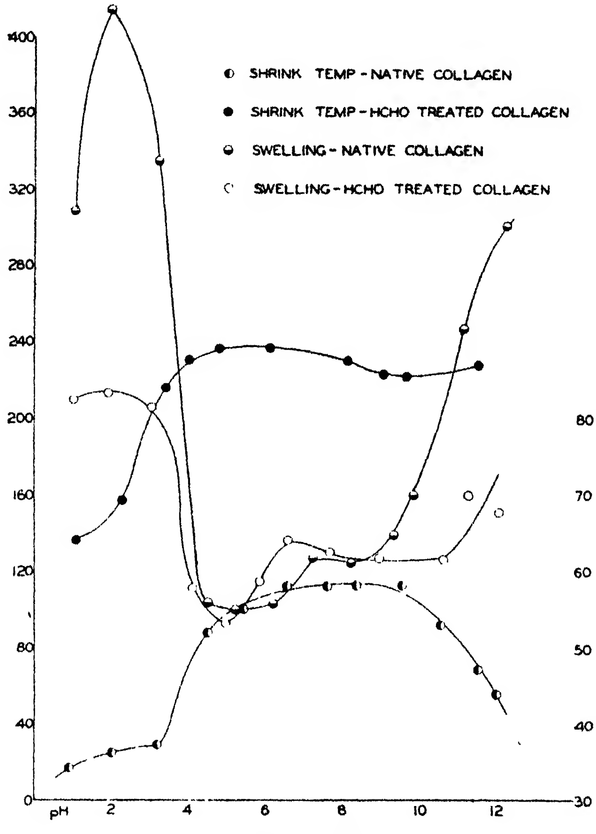


Figure 118A

by Theis and Lams relative to formaldehyde fixation show only a slight decrease in the pH zone 3.0 to 9.0. These data are in line with their suggestions that lysine plays only a small part in the pH region more acid than the isoionic point, and that formaldehyde fixation in this region is largely confined to a reaction with the least basic imino groups of the polypeptide chains. Theis and Lams believe that the slight decrease in formaldehyde binding in the acid range is probably due to two causes: (1) possible change in the

imino groups as suggested by Stiasny; and (2) the greater compactness of the fiber structure of the deaminized collagen. This contention is strengthened by a careful study of the data given by Highberger.

Effect of Temperature on Formaldehyde Fixation

Theis made a study of the effect of temperature on formaldehyde fixation by collagen and found that this factor plays a rather important role at temperatures above 20°, but that below 20° the temperature factor appeared

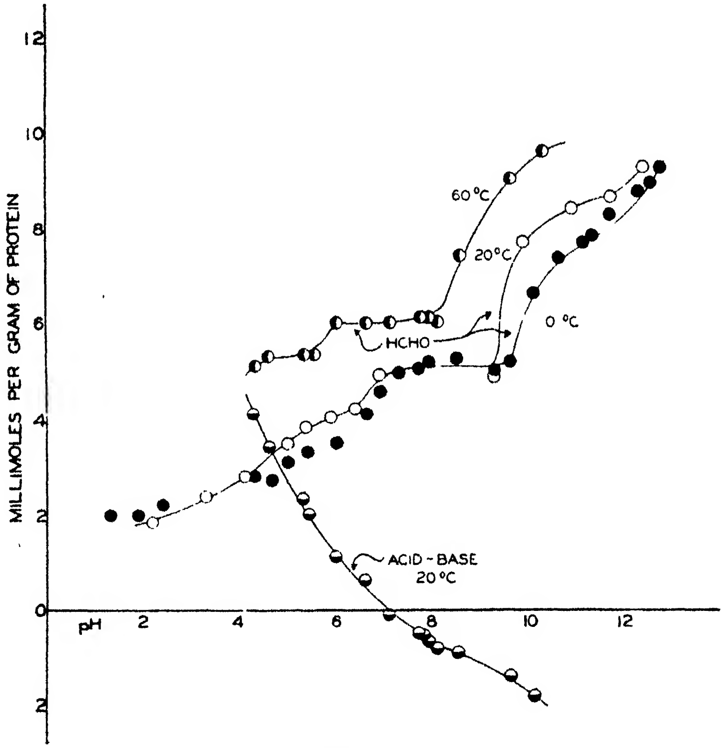


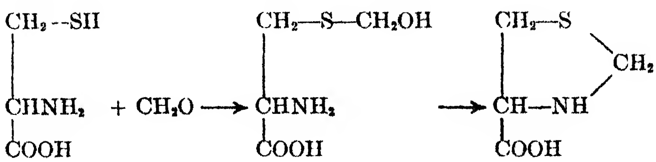
Figure 119

rather unimportant. Figure 119 pictures the formaldehyde fixation by collagen at 0°, 20°, and 60°. It can readily be seen that increased temperature reaction causes a greater amount of formaldehyde to be bound and, further, causes the plateau zone to be shifted to the left. It also appears that increased temperature acts similarly to increased formaldehyde concentration.

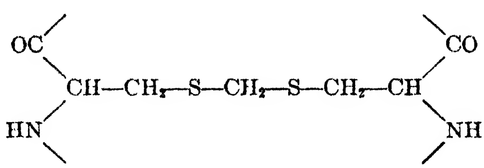
The Formaldehyde-Fixation of Keratin

The keratin proteins contain lysine, histidine, and arginine, just as in the case of collagen, but in addition, they contain tyrosine and cystine. Ratner

and Clark[22] have shown that in cystine compounds formaldehyde fixation takes place at the sulfhydryl group more readily than at the amino group. They pictured this reaction with cysteine as follows:



Ratner and Clark further claim that cystine in hair or wool is reduced to cysteine and that the sulfhydryl groups thus formed will then probably combine with formaldehyde. Without any experimental evidence, a further postulation may be made; namely, it may very well be that formaldehyde reacts to form a bridge between two cystine groups in juxtaposition:



Hegman[12] in 1942 made a study of the formaldehyde fixation of down and feather keratin. His results are shown in Figures 120 and 121. In summarizing his work, Hegman claims that the apparent aldehyde fixation by keratin at pH 1.0 to 6.0 is due to bisulfite-binding substances formed during the analysis; that at pH 6.0 to 8.0 the reaction is confined to the lysine present; that with low concentrations of formaldehyde the reaction above pH 8.0 is confined to the arginine present; and that under conditions of high pH value, the disulfide bond is broken to yield sulfhydryl groups, which in turn may bind formaldehyde.

Theis and Lams in 1942 made a comprehensive investigation of the formaldehyde-binding capacity of wool. For this study they used the unique method of pressing the wool-formaldehyde compound several times at 8-10,000 pounds per square inch pressure. This pressure, it was assumed, removed all free water, electrolyte and formaldehyde. Theis and Jacoby made use of this method in determining the acid- and base-binding capacities of fibrous proteins; these methods are fully described in Chapter 4. These investigators used a formaldehyde concentration of one per cent and a reaction time of 72 hours. Analysis of the keratin-formaldehyde compound for acid-, base-, and formaldehyde bound showed that there was no change in the isoionic point of the collagen; that the acid- and base-binding capacity of the formaldehyde-treated keratin was the same as for normal keratin in the pH range 1.0 to 9.5; that the base-binding capacity of the keratin-formaldehyde

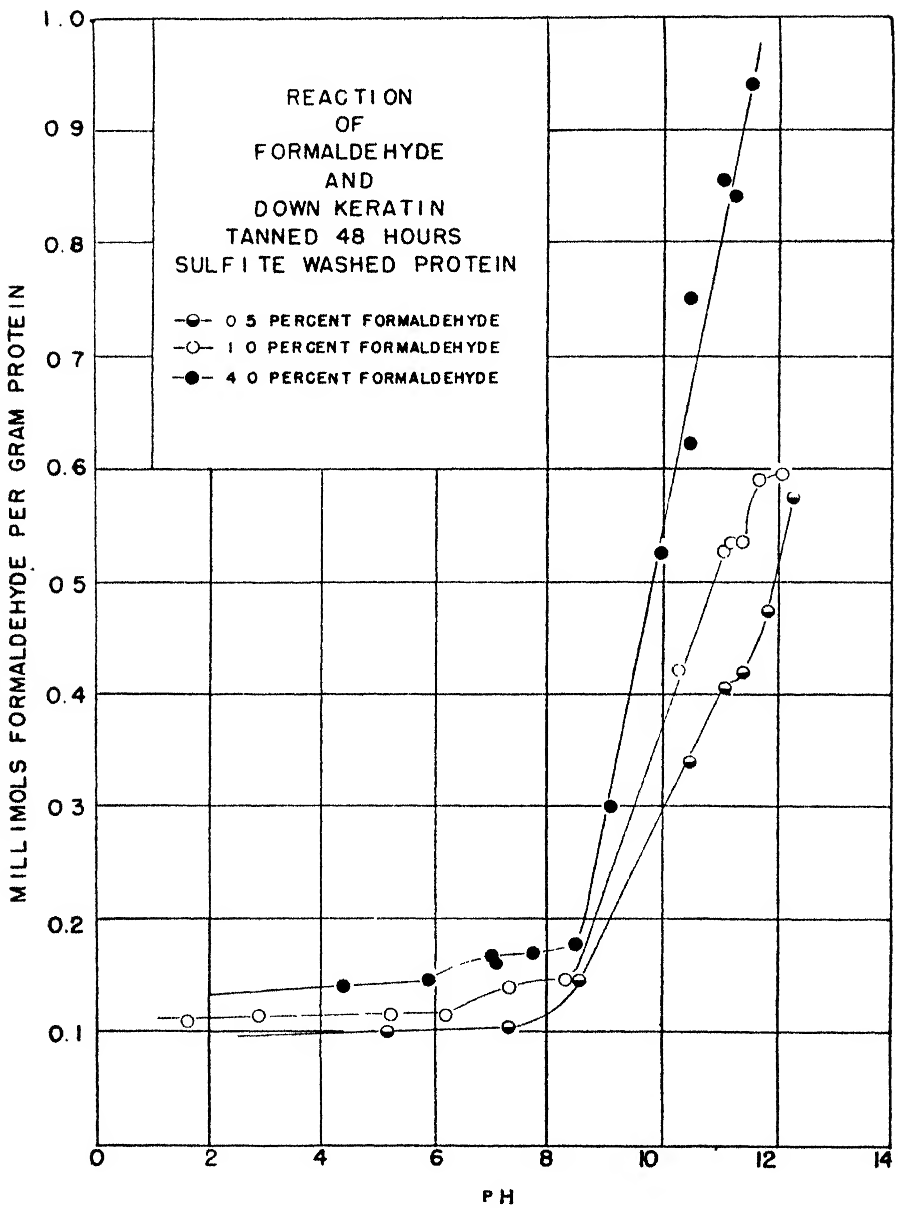

REACTION
OF
FORMALDEHYDE
AND
DOWN KERATIN
TANNED 48 HOURS
SULFITE WASHED PROTEIN
0 5 PERCENT FORMALDEHYDE
1 0 PERCENT FORMALDEHYDE
4 0 PERCENT FORMALDEHYDE
MILLIMOLS FORMALDEHYDE PER GRAM PROTEIN
1.0
0 9
0.8
0 7
0.6
0 5
0.4
0 3
0.2
0.1
0
2
4
6
8
10
12
14
PH

Figure 120

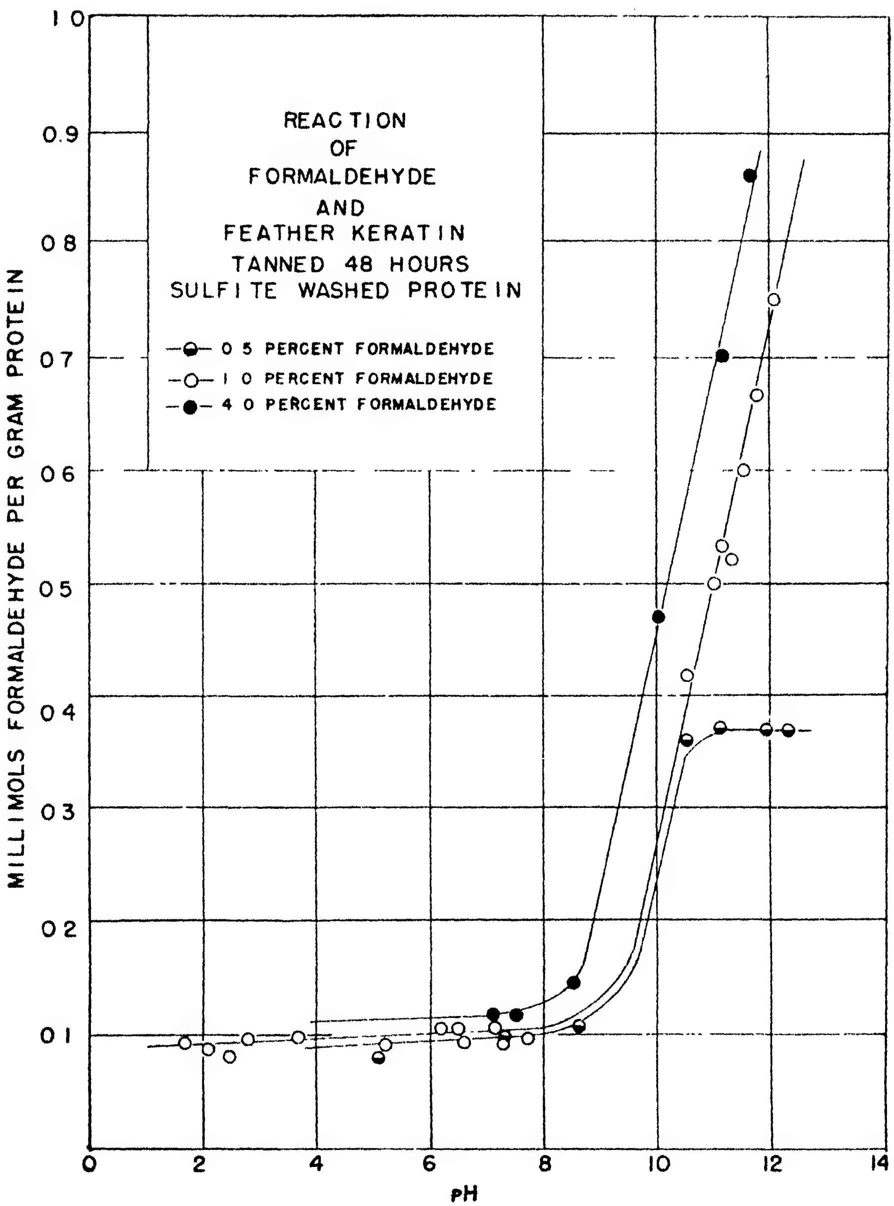

REACTION
OF
FORMALDEHYDE
AND
FEATHER KERATIN
TANNED 48 HOURS
SULFITE WASHED PROTEIN
0 5 PERCENT FORMALDEHYDE
1 0 PERCENT FORMALDEHYDE
4 0 PERCENT FORMALDEHYDE
MILLIMOLS FORMALDEHYDE PER GRAM PROTEIN
1 0
0.9
0 8
0 7
0 6
0 5
0 4
0 3
0 2
0 1
0
2
4
6
8
10
12
14
pH

Figure 121

substance was greater than that of normal keratin in the pH range 9.5 to 12.0; that in the pH range 2.0 to 6.0 some 0.3 milliequivalent of formaldehyde is fixed; that there is a definite plateau in the aldehyde-fixation curve in the pH range 8.0 to 9.5, corresponding essentially to the same type of plateau in the base-binding curve and usually interpreted as the back-titration of histidine; and that a striking increase in aldehyde bound occurs in the pH zone 9.5 to 12.5, usually attributed to the back-titration of lysine.

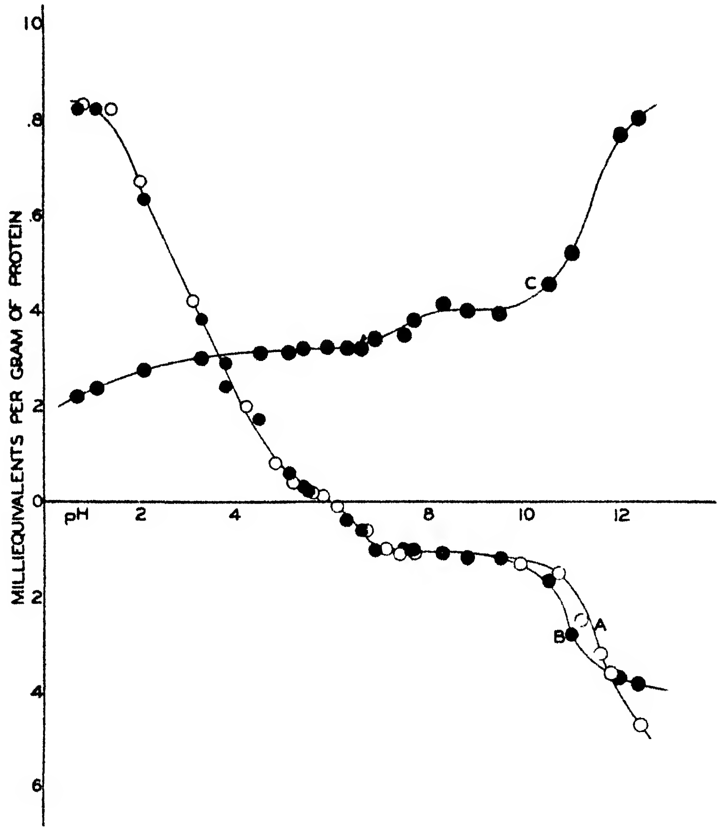


Figure 122

A careful study of the keratin-formaldehyde data of Theis and Lams brings out the striking similarity of the aldehyde-fixation and base-fixation curves. Wherever the one has a point of inflection, this same inflection obtains in the other. These workers interpret their formaldehyde-fixation curve as; an imino reaction in the pH range 1.0 to 6.0 (isoionic point), a reaction with histidine in the pH range 6.5 to 9.5, and a lysine-formaldehyde

reaction in the pH range greater than 9.5. This interpretation is in line with similar interpretations of their base-binding curves of other fibrous proteins. These investigators point out that in the pH range greater than 9.5, it is entirely possible that a second reaction occurs—namely, a reaction of formaldehyde with sulfhydryl groups, since the pK value of cysteine is 9.02. Theis and Lams state that since wool and hair keratins are very compact fibrous proteins and do not swell appreciably in the acid zone, this physical factor may account for the approximately constant aldehyde-fixation value in the acid zone. The data secured by Theis and Lams are pictured in Figure 122 and Table 154.

Table 154. The Acid, Base and Formaldehyde Fixation of Wool Keratin.

(A) Acid or Base Fixed—No CH_2O Present		Acid, Base and CH_2O Fixed		
pH	Acid* or Base	pH	(B) Acid* or Base	(C) CH_2O*
0.8	0.83	0.7	0.82	0.22
1.4	.82	1.1	.82	.24
2.0	.67	2.1	.63	.28
3.1	.42	3.3	.38	.30
4.2	.20	3.8	.24	.29
4.8	.08	4.5	.17	.31
5.2	.04	5.1	.06	.31
5.6	+.02	5.4	.03	.32
5.8	+.01	5.5	+.02	.32
6.1	−.01	5.9	.00	.32
6.3	−.04	6.3	−.04	.32
6.7	−.06	6.6	−.06	.32
7.1	−.10	6.9	−.10	.34
7.4	−.11	7.5	−.10	.35
7.7	−.11	7.7	−.10	.38
8.8	−.12	8.3	−.11	.41
9.5	−.12	8.8	−.12	.40
9.9	−.13	9.5	−.12	.39
10.7	−.15	10.5	−.17	.45
11.2	−.25	11.0	−.28	.52
11.6	−.32	12.0	−.37	.76
11.8	−.36	12.4	−.38	.80
12.4	−.47			

* Millimol per gram protein.

The Formaldehyde-Fixation of Silk Fibroin

Earlier in this chapter the authors discussed the formaldehyde-fixation capacity of wool keratin and pointed out that the trend of the fixation curve was similar to that obtained for collagen. There are certain discrepancies in the case of silk fibroin, due to the fact that the basic and acidic amino acids probably have not been determined accurately. The maximum acid-binding capacity shown by titration curves is 0.17 milliequivalent per gram, while calculated values, based upon available data for arginine, lysine, and histidine, show only 0.078 milliequivalent per gram. Such values indicate that the

data relative to these basic amino acids are undoubtedly low. There are available no definite data relating to the acidic amino acids.

Theis and Lams investigated both the acid-, alkali- and formaldehyde-binding capacity of silk fibroin. For this work, they used a specially prepared fibroin. The fibroin was studied both with and without formaldehyde over a wide pH range. A one per cent formaldehyde solution at 20° for 72 hours was used. After equilibrium had been obtained, the treated fibroin was pressed at 10,000 pounds per square inch until dry, and was then analyzed. It was assumed that the pressing removed all free electrolyte and free formaldehyde. The data taken are shown in Table 155 and Figure 123. These data

Table 155 The Acid, Base and Formaldehyde Fixation of Silk Fibroin.

(A) Acid or Base Fixed — No CH_2O Present		Acid, Base and CH_2O Fixed	(B)	(C)
pH	Acid* or Base	pH	Acid* or Base	CH_2O*
1.0	0.17	0.9	0.17	0.04
1.9	.16	2.0	.16	.04
3.1	.13	2.7	.15	.04
4.1	.11	3.3	.13	.06
4.6	.08	3.8	.11	.06
5.0	.05	4.4	.08	.07
5.6	.02	4.8	.07	.08
6.0	.00	5.3	.05	.09
6.5	−.02	5.9	.02	.09
6.7	−.04	6.1	.00	.08
7.4	−.07	6.3	−.04	.09
7.6	−.08	6.7	−.08	.10
8.3	−.09	7.0	−.09	.10
8.8	−.09	7.5	−.11	.09
9.0	−.09	8.2	−.13	.10
9.5	−.10	9.1	−.13	.11
10.2	−.14	9.9	−.11	.13
11.1	−.22	10.3	−.18	.13
11.3	−.30	10.6	.20	.14
11.6	−.35	11.3	.31	.15
12.4	−.37	11.4	−.31	.16
12.5	−.37	11.9	−.34	.16
		12.0	−.34	.16
		12.3	−.34	.16

* Millimol per gram protein.

show that formaldehyde fixation increases gradually in the pH range 2.6 to 5.3, attaining a value of 0.09 milliequivalent of formaldehyde fixed per gram at pH 5.3. From pH 5.3 to 8.2 a plateau exists, and at pH values greater than 8.2 a gradual increase in formaldehyde fixation occurs. At pH 12.0 there is an indication of a maximum value of 0.16 milliequivalent fixed per gram. Theis and Lams believe that in the range pH 8.5 to 12.0, the curve represents the reaction with the ε-amino group of lysine.

Since silk fibroin is a very compact protein and shows but little swelling at any pH value, less formaldehyde will bind at the imino groups of the polypeptide chain. However, Theis and Lams have pointed out that 0.1 millie-

quivalent of formaldehyde is bound as N--CH_2—N bridges in the pH range 5.0 to 12.0, and at pH values less than 5.0 smaller amounts are fixed in this manner. This type of fixation has been fully discussed under collagen.

Reversibility of the Protein-Formaldehyde Reaction

Salcedo and Highberger[25] in 1941 pointed out the reversibility of the protein-formaldehyde reaction. They showed that formaldehyde-treated collagen lost formaldehyde when treated with more acid buffers. Theis and

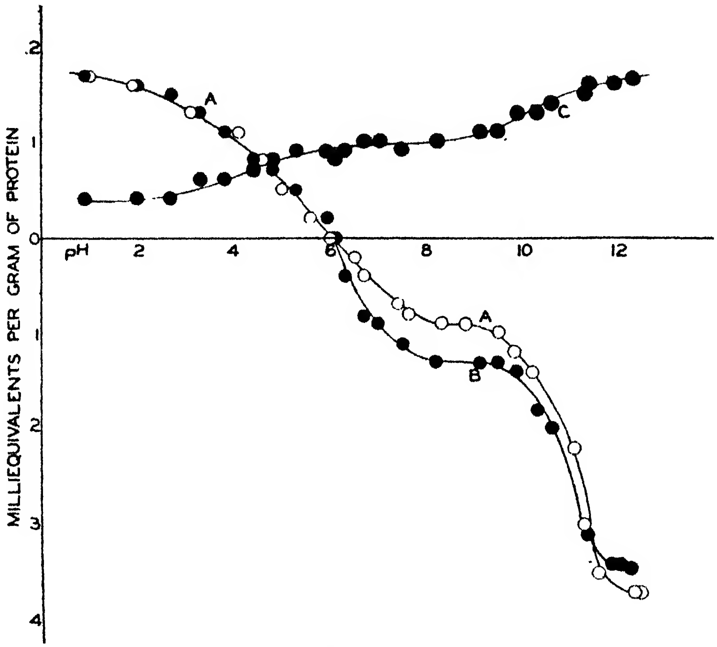


Figure 123

Lams[30] in 1943 studied the reversibility of this reaction. They found that the collagen-formaldehyde compound formed at pH 11.0 rapidly changed when placed in contact with a water solution at pH 8.0 or pH 2.0. These results are shown in Table 156.

Table 156. The Reversibility of the Collagen-Formaldehyde Reaction.

Treatment	CH_2O* Fixed	H^+ or OH^-* Bound
(1) Treated at pH 11.0 with 1% CH_2O	0.85	−0.33
(2) Same as (1) but then placed in H_2O at pH 8.0	0.47	−0.11
(3) Same as (1) but then placed in H_2O at pH 2.0	0.29	+0.94
(4) Same as (1) but then heated at 105° for 24 hours	0.70	−0.34

* Millimol CH_2O, H^+, or OH^- fixed per gram protein.

The positive reversibility of the collagen-formaldehyde reaction led the present authors to the conclusion that the formaldehyde-fixation values given by Theis and Lams, which were obtained by the pressing method, are more nearly in line with experimental facts than those given by Highberger *et al.* Theis and his students have repeatedly pointed out that the washing technique used by Highberger and others must of necessity give results not representing true facts. And on the other hand, removal of all uncombined formaldehyde by means of mere mechanical pressure insures that the fixed formaldehyde remains undisturbed.

The Effect of Denaturization

In Chapter 4 it was shown that the heat-denaturization of collagen definitely affects its ability to combine with acids and alkalies in the pH stability zone, and that the isoionic point of the denatured collagen shifts to a more alkaline point. Theis and Lams[40] investigated the formaldehyde-binding capacity of heat-denatured collagen over the pH range 1.0 to 12.0. The data obtained are shown in Table 157 and Figure 124. Whereas the formaldehyde-fixation curve for normal collagen shows a plateau in the pH range 7.0 to 9.5, the curve for the denatured protein shows a similar plateau in the range pH 4.0 to pH 7.5. Such a shift of this zone to the more acid region would seem to indicate a certain shifting of the reactive groups during the collapse of the chains upon themselves during denaturization. The whole trend of the curve appears to indicate that the acid-binding groups become stronger near the isoionic point. It also appears that in all probability the pH values of lysine and arginine are shifted to a more acid region, as evidenced by the plateau zone, by the trend of the curve in the pH range 7.5 to 11.0, and by the point of inflection at pH 11.0. From pH 2.0 to 4.0, there is a sharp

Table 157*

pH	CH_2O millimols/gram	H^+ or OH^- millimols/gram
1.2	0.28	1.17
1.9	.28	1.10
2.9	.33	0.96
4.0	.48	0.52
4.4	.50	0.44
4.8	.48	0.38
5.2	.49	0.28
5.8	.48	0.26
6.8	.49	0.10
8.5	.63	0.00
9.3	.78	−0.025
10.5	1.08	−0.045
11.0	1.15	−0.11
11.9	1.38	−0.29

* Collagen pretreated for 1 minute in water at 60° C. before placing in formaldehyde solutions.

increase in formaldehyde fixation, as is also the case from pH 7.5 to pH 11.0. With the exception of the pH range 6.9 to 7.5 the formaldehyde binding is greater at all pH values. As obtained from the acid- and base-binding curve, the denatured collagen shows an isoionic point of 8.5, indicating a positive shift to the more alkaline state.

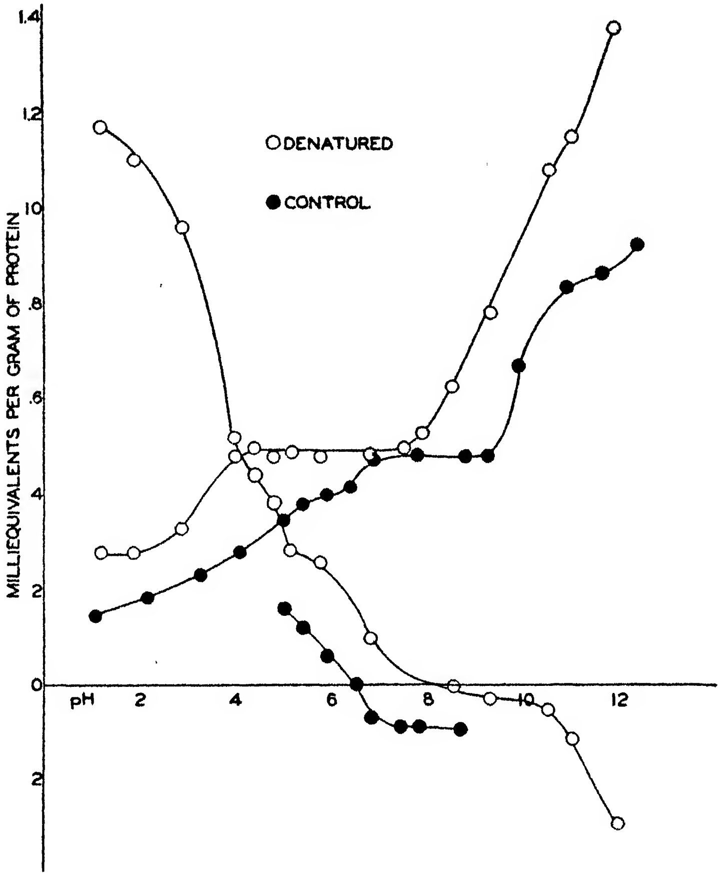


Figure 124

Formaldehyde Fixation Rate

Theis and Muraco[30] made a study of the fixation rate of formaldehyde with skin collagen. For this work properly prepared goat skin was placed in a one per cent formaldehyde solution adjusted to pH 8.0 for the time periods noted in Figures 125 and 126. At prescribed periods, pieces were removed for determination of shrinkage temperature and fixed formaldehyde. Formaldehyde was determined on the pressed sample.

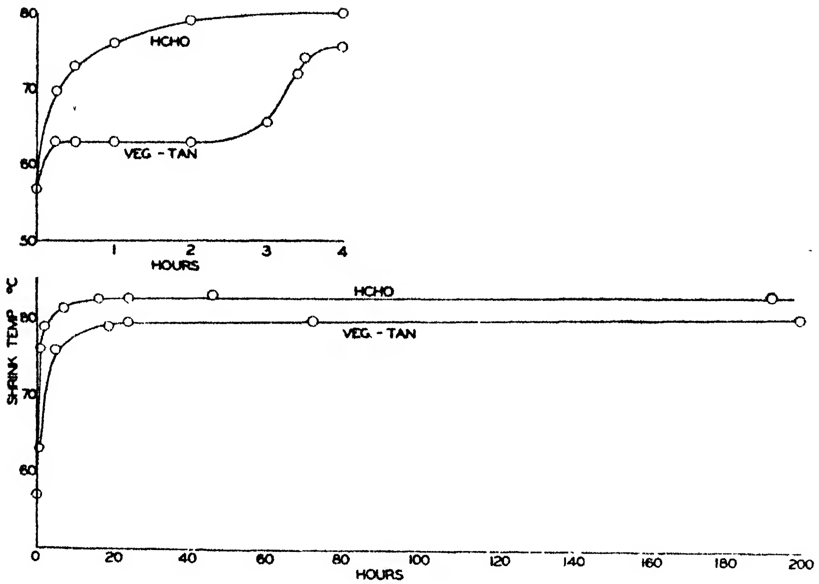


Figure 125

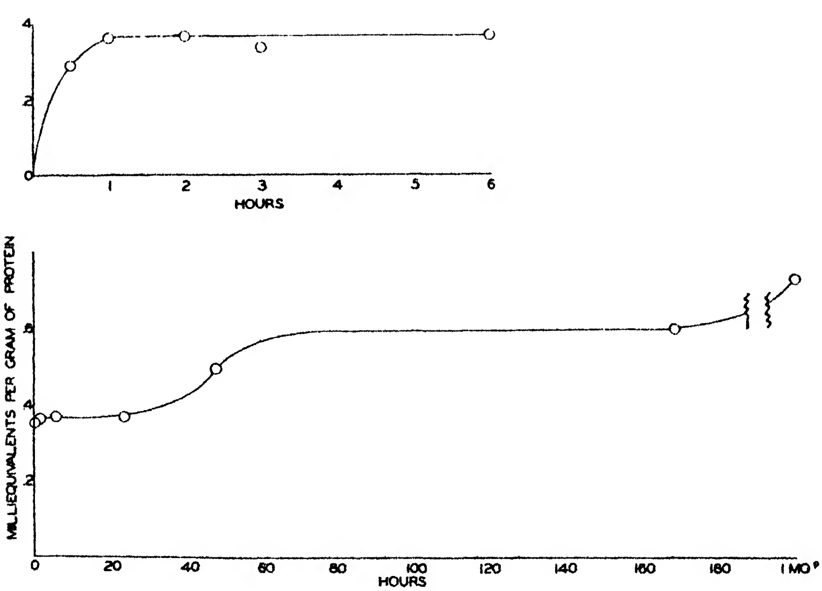


Figure 126

Figure 125 shows that the shrinkage temperature of the formaldehyde-treated skin increases sharply, attaining practically its maximum value within some 10 hours and 94 per cent of the maximum value within 4 hours. This figure compares the rate of formaldehyde tannage with that of vegetable tannage.

Figure 126 shows the rate at which formaldehyde binds with collagen. The rate of fixation is very striking during the first 30 minutes. In one hour, as much aldehyde is fixed as at 6 hours. After some 24 hours in contact with the formaldehyde solution, an increase in fixation occurs, this increase continuing for about 80 hours, after which there is only a slow increase.

Effect of Heat on Dry Formaldehyde-tanned Leather

Salcedo and Highberger[25] studied the effect of drying moist formaldehyde-treated collagen. Their collagen samples were treated with various percentages of formaldehyde at several different pH values. Instead of washing

Table 158. 2.000 Grams Collagen Powder Tanned 24 Hours in 100 ml. 0.1M Phosphate Buffer.

Grams Formaldehyde per 100 ml	Final pH	Drying Period (Hours)	Millimol Formaldehyde Fixed per Gram Collagen
1	6.46	24	0.43
1	6.46	48	0.41
1	6 46	72	0.40
1	6.46	96	0 40
1	6.46	120	0.40
2	6.42	24	0.46
2	6.42	48	0 39
2	11.33	24	0.82
2	11.33	48	0.80
4	6.02	24	0.48
4	6.02	48	0.44
4	10.55	24	0.85
4	10.55	48	0.80
4	11.74	24	0.81
4	11.74	48	0.79
4	11.74	72	0.78
4	11.74	96	0.78
4	11.74	120	0.78
8	5.92	40	0.54
8	5.92	90	0.52
8	11.17	40	0.84
8	11.17	90	0.81
16	6.10	40	0.57
16	6.10	90	0.55
16	10.78	40	0.87
16	10.78	90	0.85

the treated material at the end of the tanning period, the formaldehyde-collagen compound was dried *in vacuo* at 100°. Their data are shown in Table 158 and Figure 126A.

These investigators state that the total formaldehyde bound ranges from 0.76 to 0.89 millimol, and that there is evidence of a collagen-formaldehyde

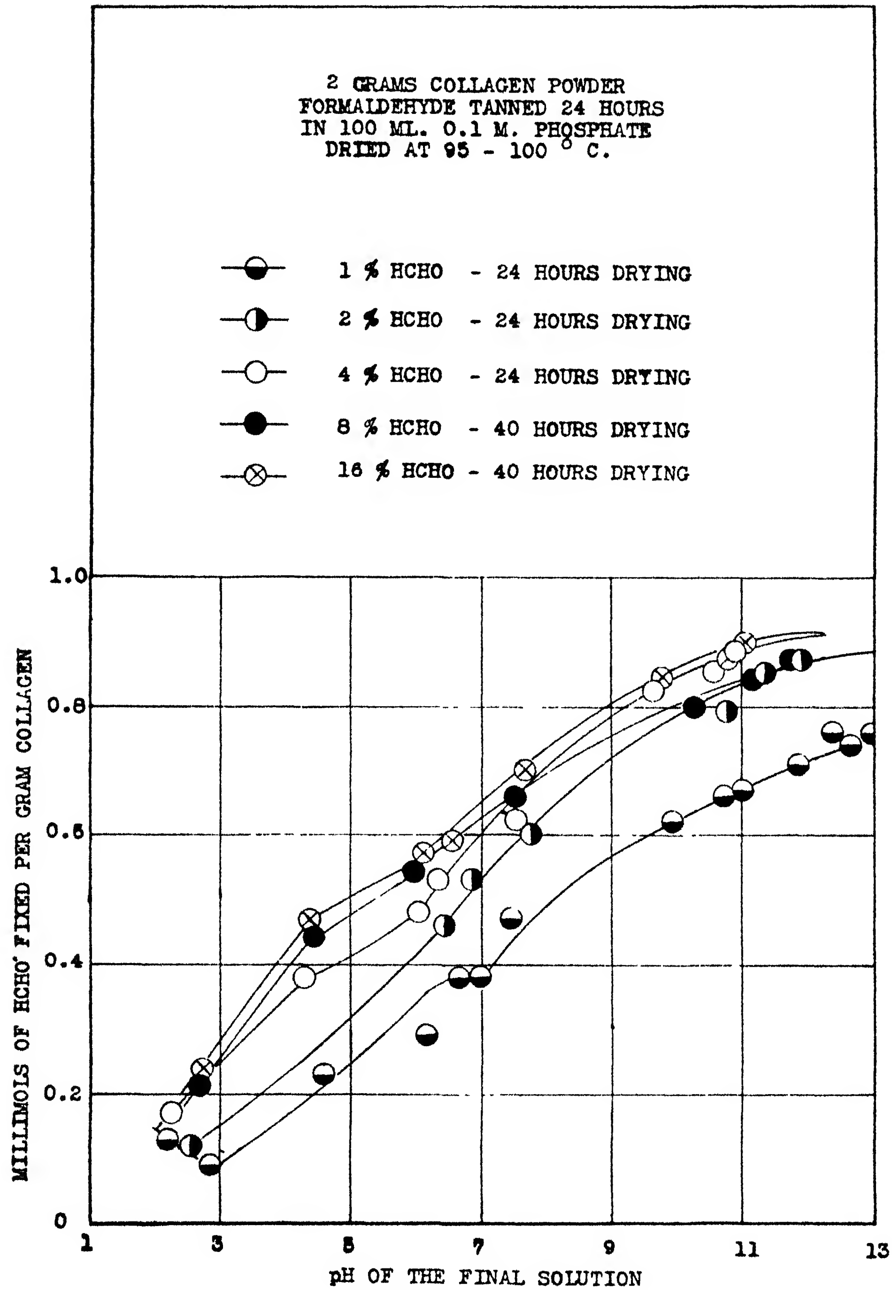

2 GRAMS COLLAGEN POWDER
FORMALDEHYDE TANNED 24 HOURS
IN 100 ML. 0.1 M. PHOSPHATE
DRIED AT 95 - 100 ° C.
1 % HCHO - 24 HOURS DRYING
2 % HCHO - 24 HOURS DRYING
4 % HCHO - 24 HOURS DRYING
8 % HCHO - 40 HOURS DRYING
16 % HCHO - 40 HOURS DRYING
MILLIMOLS OF HCHO FIXED PER GRAM COLLAGEN
1.0
0.8
0.6
0.4
0.2
0
1
3
5
7
9
11
13
pH OF THE FINAL SOLUTION

Figure 126A

reaction corresponding to a ratio of one molecule for every free amino group. They claim a heat stability factor for the resulting compound. They note the formation of a more stable compound with longer drying periods.

Theis[30] tanned collagen strips in an aqueous formaldehyde solution made 5.0 per cent with respect to formaldehyde and 0.1N with respect to potassium

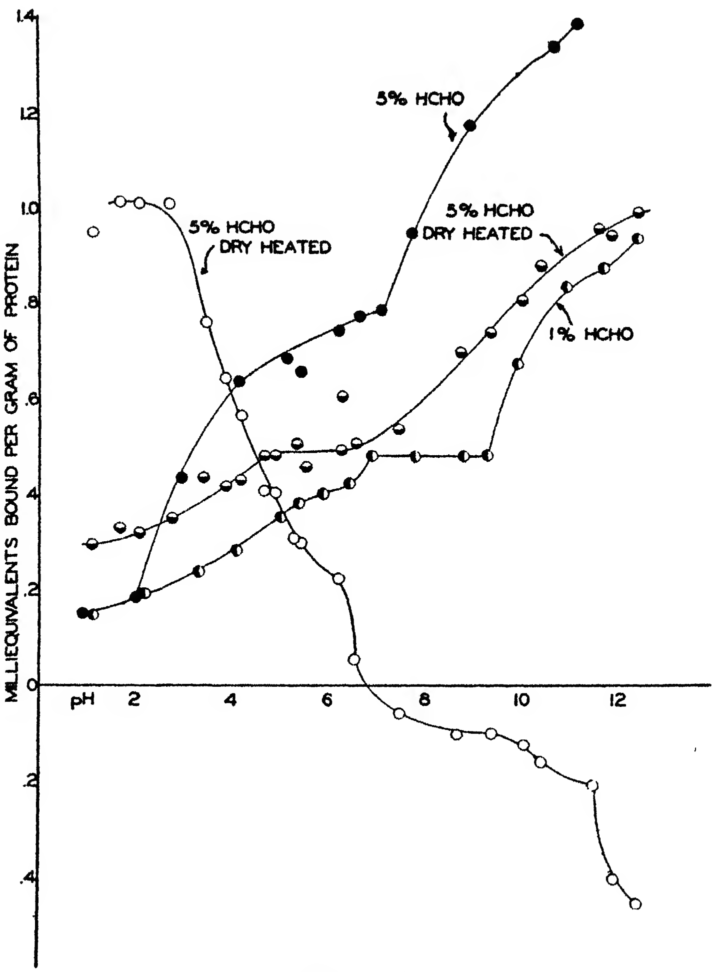


Figure 127

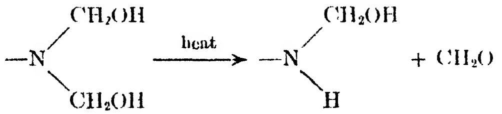
chloride. A pH range of 1.0 to 12.5 was studied. The tanning period was 72 hours at 20°, with constant agitation. After tanning, the treated collagen strips were removed, pressed twice at 10,000 pounds per square inch, and allowed to air-dry. After air-drying, one-half of each sample was dried at

105° for 48 hours. Both samples were then analyzed for formaldehyde and hide substance. The data secured are shown graphically in Figure 127.

There is little doubt that prolonged heating removes formaldehyde from the formaldehyde-collagen compound. Collagen originally treated with a 5.0 per cent solution of formaldehyde loses formaldehyde upon heating in the pH range 2.5 to 12.0. The greatest amounts of formaldehyde are lost from the collagen-formaldehyde compound in the alkaline zone. It may be stated that the curve representing a 5.0 per cent formaldehyde tannage, upon heating, attempts to correspond to that representing a 1.0 per cent formaldehyde tannage. This is especially true at pH 12.0, at which point the two curves approximately meet. The curve representing the heated collagen-formaldehyde compound has a plateau zone in the pH range 4.6 to 6.3 in contradistinction to the 1.0 per cent formaldehyde curve, which has the plateau zone in the pH range 6.9 to 9.3. The former curve shows characteristics of a denatured protein.

There is little doubt that heating of the collagen-formaldehyde compound tends to convert it to a more stable compound. However, Theis believes it is the formaldehyde attached or bound to the free amino groups that is removed and not that which has bound itself in bridge-like linkage. The heat-treated collagen-formaldehyde compound at pH 8.0 has the same shrinkage temperature as that which was not subjected to dry heat.

During the heating, it is quite possible that the formaldehyde bound to the ε-amino group of lysine is removed. This might be pictured:



Such a reaction would necessarily assume the formation of methylol groups during the tanning process.

X-ray Pattern of Formaldehyde-tanned Collagen

Katz and Gerngross[16] included formaldehyde-tanned leather in their early work dealing with x-ray studies of various tannages. At that time, however, the technique of making x-ray diffraction patterns from such substances as collagen was not sufficiently developed to permit the observation of interferences lying close to the central spot. Highberger and Kersten point out that Katz and Gerngross overlooked the pronounced loss of definition in the side chain definition which Lloyd observed for other types of leather. Katz and Gerngross observed a strengthening of the 2.8Å interference for formaldehyde-tanned leather and an unaltered collagen diagram for formaldehyde-tanned tendon collagen.

Clark and Shenk[6] in 1937 studied the x-ray diagrams given by proteins treated with formaldehyde. They found that the pattern for the gelatin-formalin compound contained several new interferences, two of which were strong rings representing spacings of 3.9 and 2.6 Å. They suggested that the appearance of these new interferences in the patterns of the gelatin-formalin

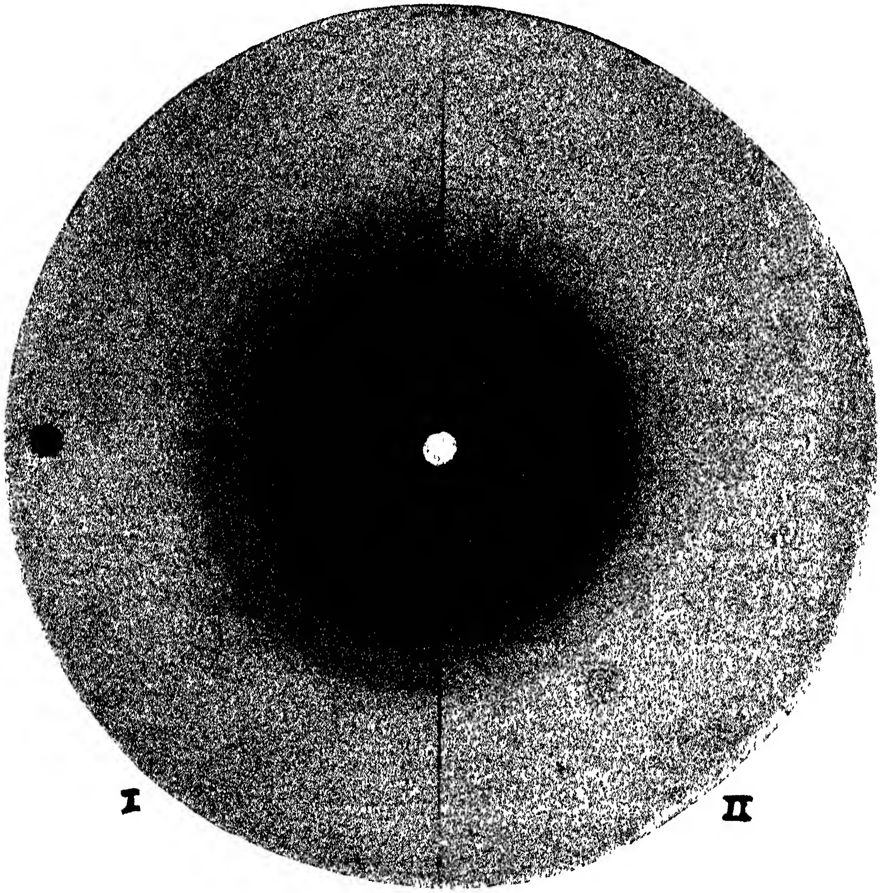


Plate 11

I. Original Collagen.
II. Collagen Tanned 48 Hours in 1 per cent Formaldehyde at pH 8.14.

compounds represented a chemical combination of the formaldehyde with the amide nitrogens of the protein chain. Highberger and Kersten questioned this hypothesis and suggested that these new interferences in the x-ray pattern might be caused by crystals of polymeric formaldehyde formed within the tendon under conditions of the experiment.

Highberger and Kersten[15] treated collagen powder with various concentrations of formaldehyde in 0.1 molar phosphate buffer for different periods of time and at pH values between 7.0 and 9.0 After treatment, the tanned collagen powder was well washed and dried *in vacuo* over P_2O_5 at room temperature. This material was used for x-ray examination. Plate 11 shows a typical example, in which the sector method is used and the pure collagen pattern is compared with that for the same collagen treated for 48 hours in a one per cent formaldehyde solution at pH 8.14. The two patterns appear to be definitely identical. Highberger and Kersten state that in no case have any of the formaldehyde-tanned collagens given an x-ray diffraction pattern in any way different from those of the original untreated collagen. They believe that the relatively simple nature of the formaldehyde molecule, when compared to the more complex structures known to exist in vegetable and basic chromium sulfate liquors, might account for the lack of alteration of the x-ray pattern.

Shrinkage Temperature of Formaldehyde-treated Collagen

In Chapter 5 the significance of shrinkage temperature measurements was discussed in detail. In this discussion it was pointed out that swelling and dehydration played an important role in evaluating this factor.

Theis and Muraco[30] investigated the relation of swelling to shrinkage temperature of formaldehyde-treated collagen and their results are shown in Figure 118A. This figure shows the swelling and shrinkage temperature of both native and aldehyde-treated collagen. It is interesting to note that formaldehyde retards swelling in the pH range 4 to 12, and especially at pH values greater than 8. It is in the pH range 4 to 10 that the shrinkage temperatures of the collagen-formaldehyde compound are definitely increased. This figure substantiates data given in a previous chapter; namely, that wherever swelling occurs the cohesive forces of the collagen are weakened, and thus the protein shows a lower shrinkage temperature.

Theis and Esterly[30] studied the effect on formaldehyde tannage of such salts as potassium and calcium chlorides. These investigators studied comprehensively the effect of these salts in a one per cent formaldehyde solution over the pH range 1 to 12. Their data as regards the shrinkage temperature of the collagen-formaldehyde compound are shown in Figures 128 and 129. Figure 128 shows the effect of calcium chloride concentration upon the shrinkage temperature of native collagen and indicates rather definitely that this particular salt drastically affects the structural cohesive forces of the protein. This fact has been pointed out in Chapter 5. Figure 129 shows the effect of salt pretreatment of collagen on the shrinkage temperature of formaldehyde-tanned collagen. This figure gives two sets of data: (A) the one showing a comparison of the effects of $0.1N$ potassium chloride and of calcium chloride.

Part A of this figure shows that in the pH range 5 to 8 the shrinkage temperature is just about the same for water and the two salts; however, at pH values 9 to 11 potassium chloride gives a slightly higher shrinkage temperature than does water, but calcium chloride gives a decided increase in this factor. It

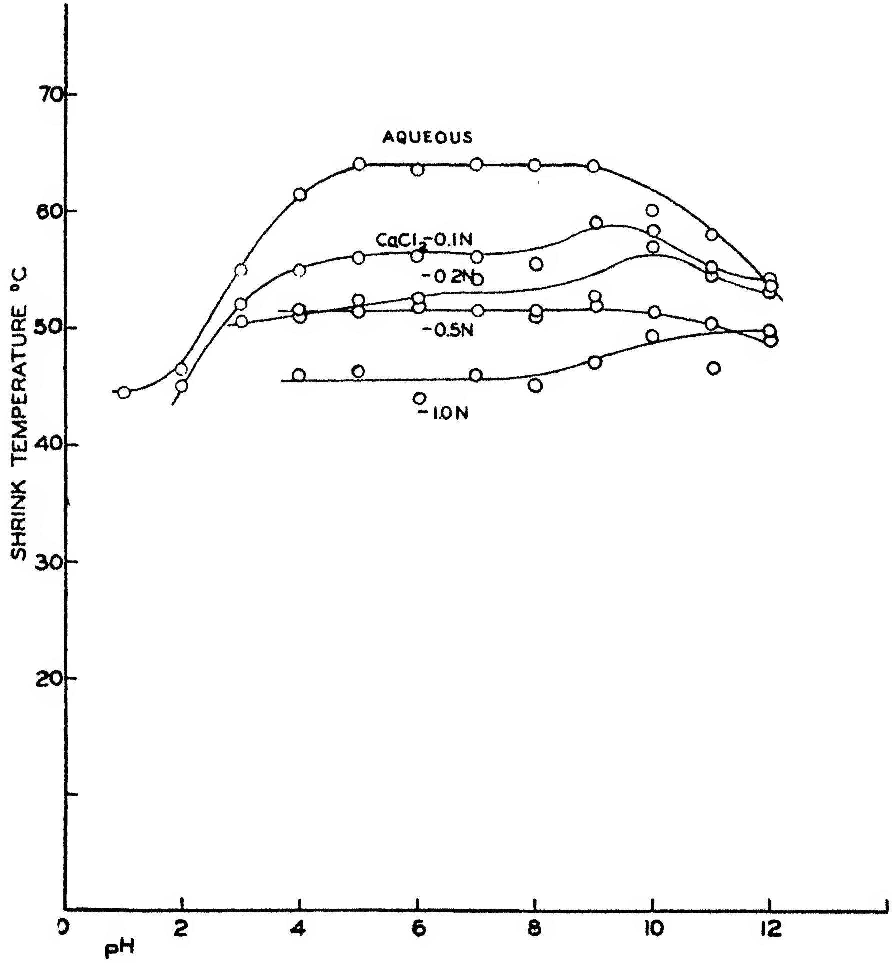


Figure 128. Effect of Calcium chloride treatment at various pH values upon shrinkage temperature of collagen.

may be said that the curves representing data taken for water or potassium chloride solutions are practically the same, but that the curve for dilute calcium chloride shows increased structural stability in the alkaline zone. This is in line with previous statements that in the alkaline region calcium

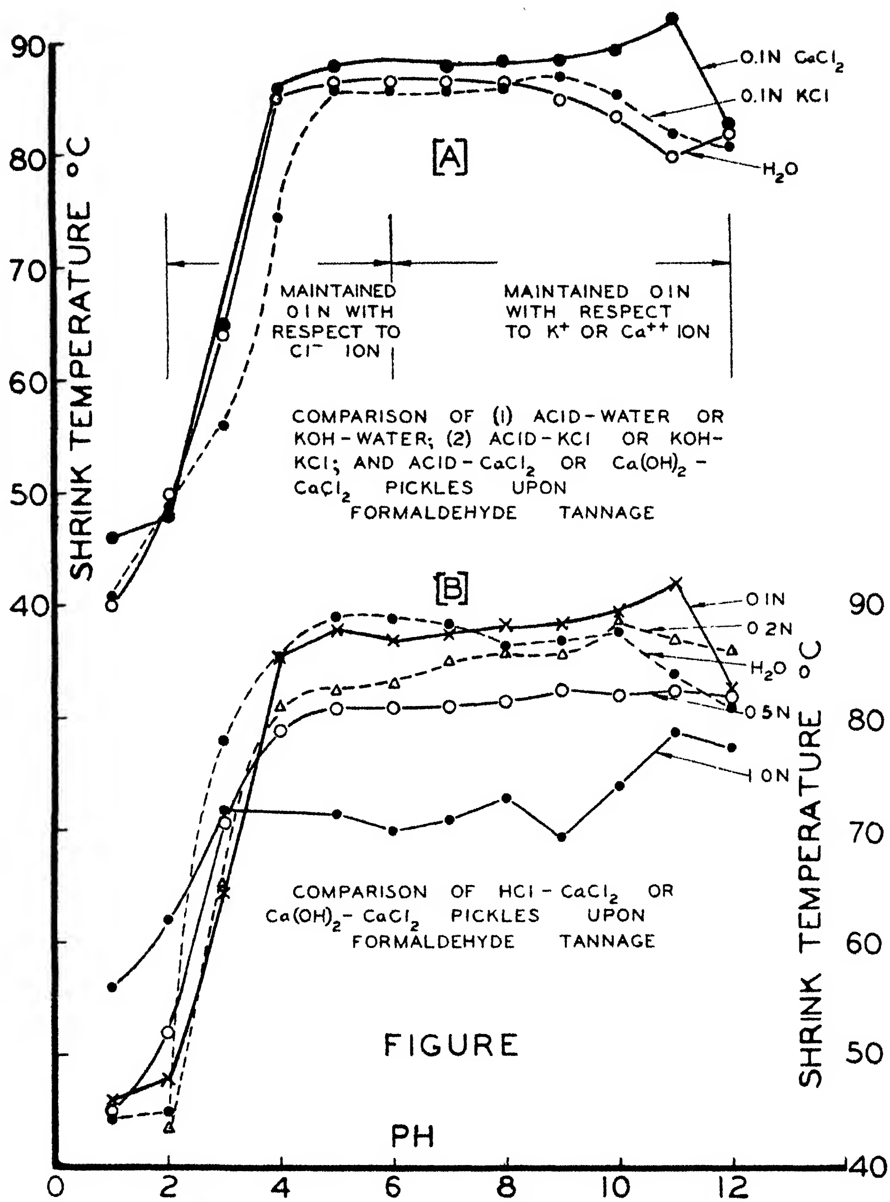


Figure 129. Effect of salt pretreatment of collagen upon shrinkage temperature of formaldehyde-tanned collagen.

chloride causes decidedly more dehydration than sodium chloride, giving a pickling effect, and therefore this increase should manifest itself in increased structural stability after formaldehyde fixation.

Part B of Figure 129 shows the effect of the more concentrated solutions of calcium chloride upon the subsequent shrinkage temperatures of the formaldehyde-treated skin. The curves show definitely that the structural breakdown caused by the concentrated calcium chloride solutions manifests

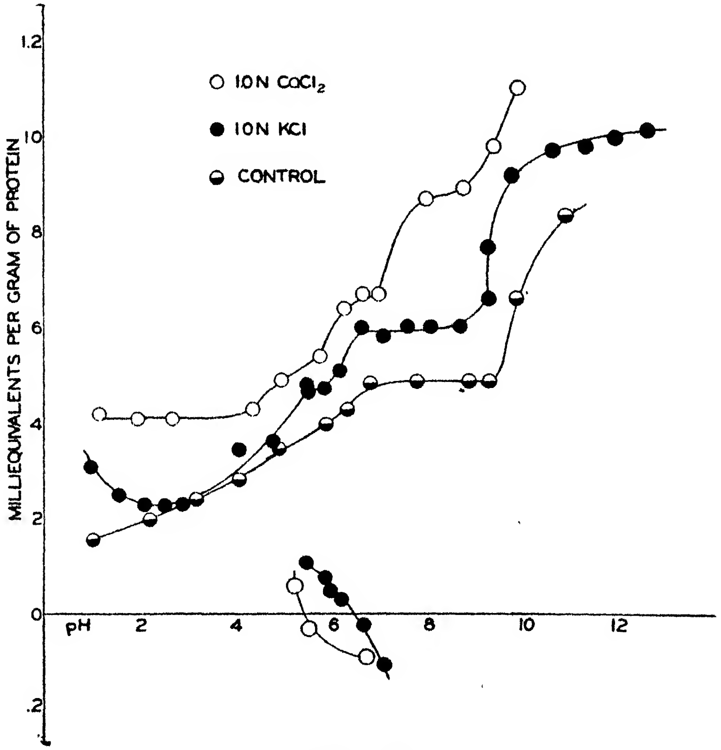


Figure 130

itself and persists even after formaldehyde treatment; in other words the real break-down of the carbonyl-imino linkages cannot be reversed. This is very evident in calcium chloride solutions having a strength greater than 0.2N. In the isoelectric zone pH 4 to 8 the decreased shrinkage temperature of the formaldehyde-treated skin as calcium chloride increases is very marked.

Undoubtedly pretreatment with calcium chloride causes a definite weakening of the collagen structure, and this weakening maintains itself even during the formaldehyde treatment. The structural break-down however, does not

interfere with formaldehyde fixation, as is evident from Figure 130, which shows in no indefinite manner that calcium chloride not only shifts the isoionic point but definitely increases the formaldehyde fixation over the entire pH scale. Potassium chloride does not affect the isoionic point but does increase

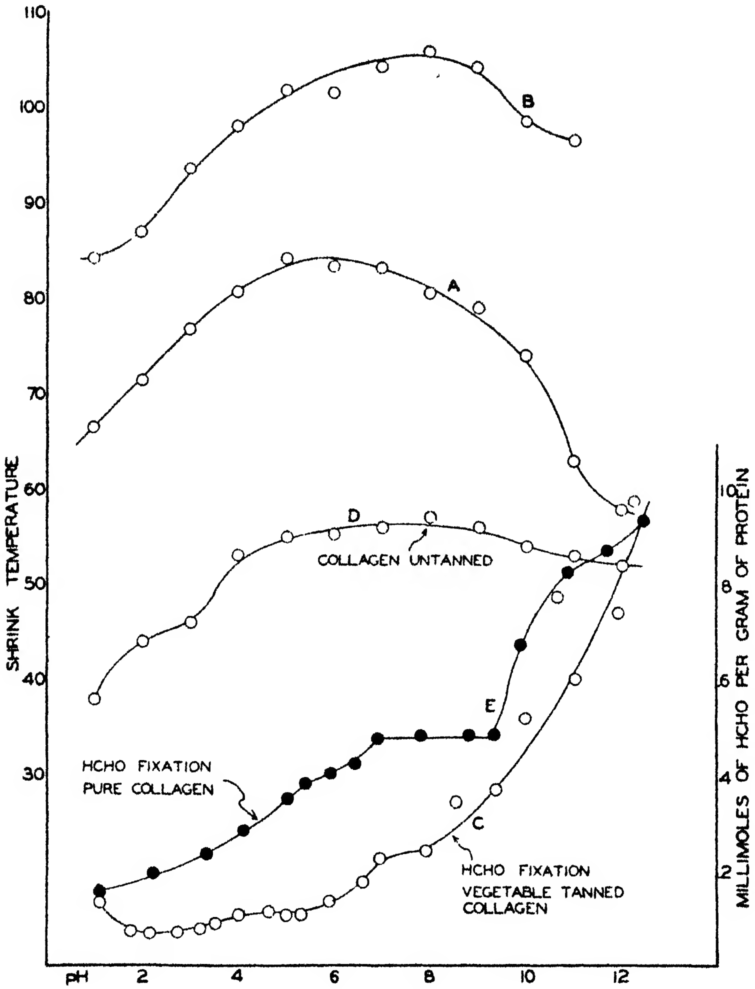


Figure 131

the formaldehyde fixation. In this case, however, the collagen-formaldehyde compound is structurally stable and resembles that compound formed without the added salt. Figure 130 definitely proves that the amount of formaldehyde fixed has little bearing upon the degree of "leathering" as measured by

the shrinkage temperature and, further, that it is the manner in which the formaldehyde binds that determines the structural strength of the collagen-formaldehyde compound.

Formaldehyde Retannage

Theis and Blum[30] found that treating vegetable-tanned leather with formaldehyde gave a much increased thermolability as measured by the shrinkage temperature. They postulated that the reaction might be an additive one, or that the formaldehyde possibly condensed with the tannin molecule, giving rise to a different molecule.

In a further investigation, Theis and Friedman[30] studied the formaldehyde fixation of thoroughly washed vegetable-tanned collagen over a wide pH range. They found in the pH range 1.0 to 8.0 that the formaldehyde fixation was less than half that of native collagen. However, in the pH range greater than 10.0, the formaldehyde fixation approached that normally obtaining for native collagen. These data are shown in Figure 131. This figure shows: (a) the shrinkage temperature of untreated, washed, vegetable-tanned leather; (b) the shrinkage temperature of formaldehyde-treated vegetable-tanned leather; and (c) the formaldehyde fixation of the washed and treated vegetable-tanned leather.

These data would appear to indicate and to substantiate further the suggestions of Theis that formaldehyde, in the pH range less than that of the isoionic point, combines with the weakly basic imino groups of the polypeptide chains in a bridge-like manner: $N—CH_2—N$. In this particular range undoubtedly the charged amino groups $-NH_3^+$ have been mostly satisfied by the acidic tannin molecules $—NH_3^+ \, T^-$. *A priori*, such a suggestion would indicate that vegetable tannin molecules also bind with the imino groups in such a manner that the tannin molecule is in coordinate linkage with imino groups in juxtaposition. This suggestion is substantiated by the fact that only very small amounts of formaldehyde are fixed in the pH range 2.0 to 6.0, which would not be the case if the complex tannin molecule were not bound with certain of the weakly basic imino groups of the polypeptide chain. In the more alkaline range, the formaldehyde bound approaches that normally bound by native collagen. This is what might be expected, since in this region little tannin is bound other than that held by the imino groups and the formaldehyde then binds with the $—NH_2$ groups of lysine, giving rise to the same condition that exists for native collagen.

Formaldehyde retannage of chrome- and alum-tanned leather results in a finished leather of increased structural stability. In the case of chrome-tanned leather, this increase in shrinkage temperature is not as striking as that noted for formaldehyde-retanned, vegetable-tanned collagen.

Theories of Formaldehyde Tannage

The great majority of the investigators studying the protein-formaldehyde reaction have not in the main been interested in other than theoretical formaldehyde fixation. Stiasny[28] was probably the first worker in this field to postulate the reaction taking place as applied to formaldehyde tanning. He suggested that two reactions are involved, the one a rapid binding with amino groups and the other a slow fixation with the peptide groups. Dyachenko and Shelpakova[8] suggested the formation of methylene bridges between amino groups in juxtaposition in practically neutral solutions. Küntzel[16a] postulated the formation of bridges between imino groups of the polypeptide chains. Holland[15a] states that the reaction is not that postulated by Küntzel, but may possibly be a reaction with acid amide groups. Theis and Schaffer[30] early suggested that the reaction which effects the structural stability is a very rapid one. This work was substantiated by Casaburi and Cantarella.[6a] Theis *et al.* suggested that the reaction postulated by Küntzel probably fits the experimental facts more closely than any other theories advanced to date. Bowes and Pleass[5] advance the same suggestions as Highberger *et al.*[14] with regard to the function of the imino groups of the polypeptide chain, claiming little fixation of formaldehyde with these groups.

Theis *et al.* from 1936 on have advocated the idea that it is the formation of bridges between adjacent polypeptide chains which is responsible for the drastic increase in thermolability as measured by shrinkage temperature. Their work in 1942-43 has further borne out this contention. Their latest work[30] shows rather definitely that the reaction, accounting for the increase in structural stability, is a very rapid one and is practically independent of the pH at pH values greater than 4.0. This reaction is the one responsible for the tannage obtained. At pH values greater than 9.0, a second reaction occurs, namely, a binding of formaldehyde with the ϵ-amino group of lysine. This fixation, however, has little to do with the structural stability. Using more concentrated formaldehyde solutions, it was found that increased formaldehyde concentration did not affect the structural stability at pH values greater than 8.0.

Theis *et al.* explain their findings as a binding of formaldehyde with some of the imino groups of the peptide chain in a bridge-like linkage over the pH range 3.0 to 11.0 and more or less independent of the pH in this range. This type of formaldehyde-fixation is rapid and is responsible for the increased shrinkage temperature of formaldehyde-treated collagen. At pH values greater than that obtaining at the isoionic point, a second reaction occurs: namely, a binding of formaldehyde with the more basic groups of lysine. The suggestions of Theis *et al.* are in accord with those expressed by Küntzel; but in this case, experimental data had been given in support of the theory advanced. Theis *et al.*, while suggesting the bridging of polypeptide chains

by formaldehyde reaction, point out that the future may give us additional data pointing to a different structural arrangement, but maintain that at this time it is the most logical explanation of the facts brought out by scientific investigations.

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Chapter 13

Quinone Tanning

In 1908-09, Meunier and Seyewetz first pointed out that *p*-benzoquinone had rather remarkable tanning properties. The leather resulting from the fixation of the *p*-benzoquinone by animal skin has a high shrinkage temperature and water resistance. But the difficulty of manipulation, low weight gain and production expense has retarded the commercial exploitation of quinone leather and even its scientific investigation.

Many investigators have studied in a comprehensive manner the reaction between the various aldehydes and collagen, and these studies have been of immense value in postulating certain theories of tanning. Like formaldehyde, *p*-benzoquinone has a relatively low molecular weight and, further, as it is initially used, it has a rather well defined structure. It therefore should be an excellent reagent for study in its reaction with collagen.

In 1924-26, Thomas and Kelly[7] and Thomas and Foster[8] studied the reaction between collagen and *p*-benzoquinone. Since the investigations of Thomas and Kelly have been extensively discussed in Volume II of the second edition of this monograph, only certain "highlights" of their work will be dealt with here. These investigators found a characteristic and striking minimum fixation by hide powder at pH 9.0. At the time of their investigations no accurate method for measuring pH values of quinone solutions was available and therefore their results, interpreted in the light of modern methods of determining pH value, require some modification. The data of Thomas and Kelly in 1924 are shown in Table 159.

Table 159. Fixation of Quinone in 24 Hours, 2 Weeks, and 5½ Weeks. Increase in Weight of 2 Grams Dry Hide Powder.

No.	pH	24 Hours	2 Weeks	5½ Weeks
1	5.0	0.056	0.908	1.089
2	6.0	0.170	0.868	1.028
3	7.0	0.540	0.915	0.980
4	8.0	0.587	0.947	0.964
5	8.5		0.919	0.860
6	9.0	0.607	0.811	0.851
7	9.5	0.596	0.897	0.973
8	10.0	0.658	0.938	0.966
9	11.0	0.636	0.893	0.937
10	12.0	0.416	0.587	0.617
11	13.0	Loss of .303	Discarded	Discarded

Stecker and Highberger[4] have pointed out that certain changes in the pH values of these experiments may have occurred due to (a) solution of the quinone in the particular buffer and (b) adsorption of the buffer components by the hide powder. Such changes in the pH values might give a somewhat

Table 160. Weight Increase of Collagen Powder in Phosphate-Buffered Quinone Solution. (2.000 gms Collagen in 200 ml Solution) Purified Collagen

Initial pH	Final pH	Weight Increase	Initial pH	Final pH	Weight Increase
	(6 hours)			(24 hours)	
5.0	5.0	0 020	5.0	5.0	0.042
6.0	5.9	0.055	6.0	5.9	0.100
7.0	6.8	0 280	6.5	6.4	0.500
8.0	7.3	0.440	7.0	6.8	0.658
9 0	7.5	0.425	8.0	7.3	0.662
10.0	7.6	0.450	9.0	7.5	0.640
10.5	8.0	0.445	10.0	7.6	0 665
10.8	8.7	0.320	10.5	8 0	0.700
11.0	9.0	0.242	10.8	8.7	0.585
11.6	9.5	0.100	11.0	8.9	0.405
			11.0	8.9	0.405
			11.6	9.5	0.150
			12.0	10.0	0.050
	(48 hours)			(1 week)	
5.0	5.0	0.100	5.0	4.6	0.450
6.0	5.9	0.210	6.0	5.9	0.775
7.0	6.8	0 838	7.0	6.7	0.950
8.0	7.3	0.840	8.0	7.3	0.965
9.0	7.5	0 805	9.0	7 5	0.880
10 0	7 55	0.807	10.0	7.6	0.982
10.5	8.0	0.870	10.5	8.0	1.015
10.8	8.7	0.750	10 7	8.5	0.915
11 0	8.9	0.520	10 8	8.7	0.700
11.6	9.5	0.150	11.0	8.9	0.410
			11.6	9.5	0.150
			12.0	10.0	0.050
	(2 weeks)			(5 weeks)	
5.0	4.1	0.998	5.0	4.0	1.160
6.0	5 6	0.910	6 0	5.55	1.075
7.0	6.8	1 002	7.0	6.7	1.045
8.0	7.3	0.968	8 0	7.2	1.005
9.0	7.4	0.885	9.0	7.4	0.925
10.0	7.5	0.880	10 0	7.5	0.902
10.5	8.0	1.012	10.5	8 0	1.015
10.8	8.7	0.925	10 8	8.65	0.945
11.0	8.9	0.520	11.0	8.9	0.520
11.6	9.5	0.150	11.6	9.5	0.150

different shape to the curve, or at least a shift in its position when accurate pH measurements of the quinone hide powder system were obtained.

Stecker and Highberger[4] in 1942 made a study of the quinone-collagen reaction and repeated, in the main, the work of Thomas and Kelly; but in their investigation measurements of the pH value of the systems were obtained

Table 161. Weight Increase of Hide Powder in Phosphate-Buffered Quinone Solution (2.000 gms Hide Powder in 200 ml Solution) Regular Collagen

Initial pH	Final pH	Weight Increase	Initial pH	Final pH	Weight Increase
	(24 hours)			(1 week)	
5.0	5.0	0.021	5.0	4.4	0.460
6.0	6.0	0.086	6.0	5.6	0.730
7.0	6.7	0.530	7.0	6.8	0.972
8.0	7.0	0.575	8.0	7.3	0.935
9.0	7.5	0.600	9.0	7.4	0.910
10.0	7.6	0.670	10.0	7.5	0.870
10.5	8.2	0.719	10.5	7.6	0.960
10.8	8.8	0.615	10.8	8.0	0.952
11.0	9.1	0.350	11.0	8.8	0.400
11.6	9.5	0.150	11.6	9.5	0.150
12.0	10.0	0.050			
	(2 weeks)			(5 weeks)	
5.0	4.1	0.980	5.0	4.0	1.150
6.0	5.6	0.830	6.0	5.5	1.049
7.0	6.8	0.989	7.0	6.7	1.020
8.0	7.3	0.990	8.0	7.2	0.983
9.0	7.5	0.870	9.0	7.5	0.900
10.0	7.7	0.973	10.0	7.7	0.973
10.5	8.0	0.995	10.5	8.0	0.995
10.8	8.7	0.945	10.8	8.7	0.945
11.0	8.9	0.600	11.0	8.9	0.600
11.6	9.5	0.150	11.6	9.5	0.150

Table 162. Weight Increase of Collagen Powder in Borate-Buffered Quinone Solution. (2.000 gms Collagen in 200 ml Solution)

Initial pH	Final pH	Weight Increase	Initial pH	Final pH	Weight Increase
	(24 hours)			(48 hours)	
5.8	4.8	0.056	6.5	5.0	0.097
7.5	6.0	0.085	7.5	6.0	0.175
8.0	7.4	0.355	8.0	6.5	0.382
8.5	8.0	0.552	8.5	7.0	0.598
8.75	8.4	0.515	8.75	8.4	0.515
9.0	8.8	0.440	9.0	8.8	0.485
	(1 week)			(2 weeks)	
5.8	3.6	0.335	5.8	3.1	0.675
6.5	3.9	0.390	6.5	3.2	0.755
7.5	4.8	0.725	7.5	4.0	1.175
7.75	4.9	0.750	7.75	4.8	1.298
8.0	5.8	0.998	8.0	5.6	1.155
8.5	6.6	0.800	8.5	5.8	1.055
9.0	7.6	0.650	9.0	6.4	0.825
10.7	8.9	0.442	9.75	7.5	0.637
			10.7	8.3	0.495
			10.9	8.6	0.458
			11.3	8.9	0.420

with a glass electrode assembly. These investigators used saturated quinone solutions, prepared by dissolving 2.74 grams of quinone in 200 ml of phosphate or boric acid buffer solution. To these portions of solution were added 2

grams of collagen. Tannage was carried out at room temperature, usually with intermittent shaking. At the end of the tanning period, the equilibrium pH value of the solution was obtained and the quinone-tanned collagen was

Table 163. Weight Increase of 2 Grams Collagen Powder Tanned for 24 Hours in 200 Ml Phosphate-Buffered Quinone Solution.
(Solution Aged 2 Weeks before Use)

Initial pH	Final pH	Weight Increase
5.0	4.2	0.347
6.0	5.7	0.379
7.0	6.8	0.594
8.0	7.2	0.489
9.0	7.3	0.502
10.0	7.5	0.515
10.8	7.7	0.457
11.6	8.0	0.298
12.0	8.5	0.100

Table 164. Weight Increase of Collagen Powder in Phosphate-Buffered Quinone Solution Containing 1.5% H_2O_2
(2.000 gms Collagen in 200 ml Solution)

Initial pH	Final pH	Weight Increase
2.0	1.9	0.292
3.9	2.9	0.840
5.0	3.0	0.886
5.5	3.4	0.984
6.0	3.7	0.972
6.3	4.0	0.660
6.7	5.0	0.364
7.0	5.2	0.250
7.2	5.4	0.204
8.0	5.6	0.064
8.7	5.8	0.004
10.0	5.9	0.010
11.0	6.6	0.005

Table 165. Effect of pH on Oxidation of Quinone During 24-Hour Oxidation Period.
(0.1 Gram Quinone in 200 ml)

Phosphate Buffer			Borate Buffer		
Initial pH	Final pH	Ml 0.8N $KMnO_4$	Initial pH	Final pH	Ml 0.8N $KMnO_4$
2.0	2.0	0.0	3.7	3.7	0.0
6.0	6.0	0.6	5.3	5.3	0.4
7.2	7.1	3.2	6.7	6.6	0.5
7.7	7.4	4.5	7.0	6.9	0.5
8.0	7.7	1.9	7.5	7.2	3.6
8.1	7.8	2.6	7.6	7.4	5.0
8.7	8.1	6.1	8.0	7.6	6.1
10.0	8.3	8.0	8.1	7.7	6.4
10.2	8.7	9.6	8.2	8.0	7.6
10.7	10.0	11.2	8.5	8.3	8.3
			9.3	9.1	9.5

washed thoroughly with distilled water in Wilson-Kern extractors. After washing, the collagen was air-dried, then dried *in vacuo* at 110° and weighed. Their data are shown in Tables 160-165 and in Figures 132 to 137.

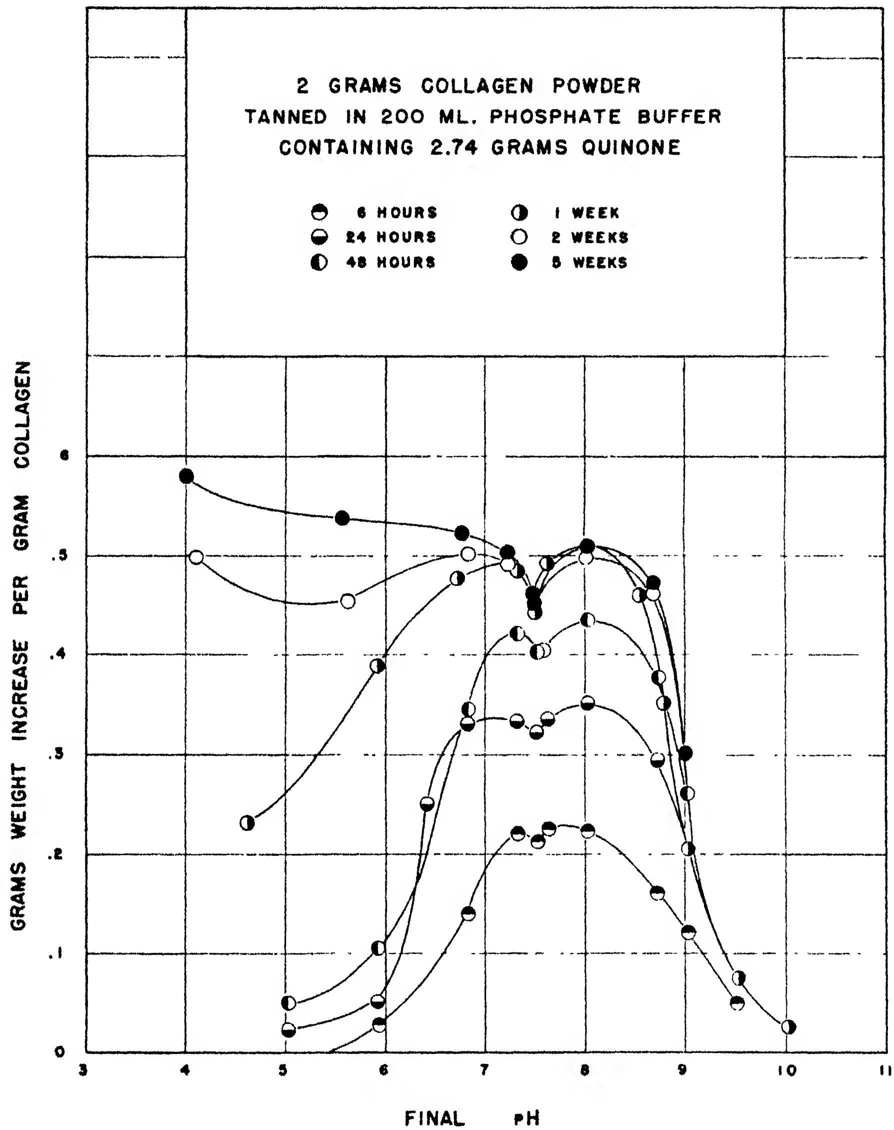


Figure 132

Stecker and Highberger considered the weight increase of the quinone-collagen compound over that of the original collagen as the amount of quinone fixed. This fixation, as determined by weight increase, for the phosphate buffer system definitely shows a minimum fixation point at about pH 7.5.

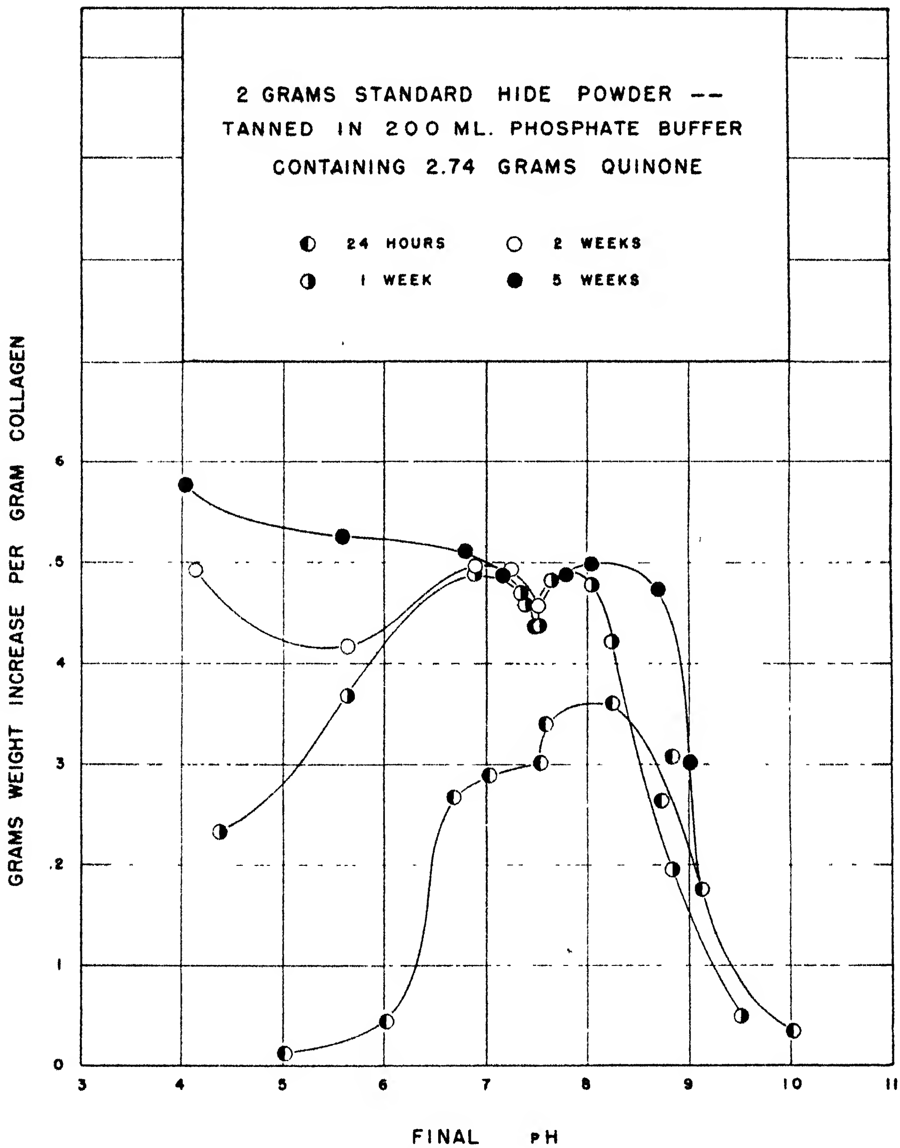


Figure 133

Stecker and Highberger first believed that this minimum value might be due to, or related to, the isoelectric point of the purified collagen used by them in their first studies. However, subsequent investigation, using regular hide powder (the isoelectric point of which is about pH 5.0) showed this minimum

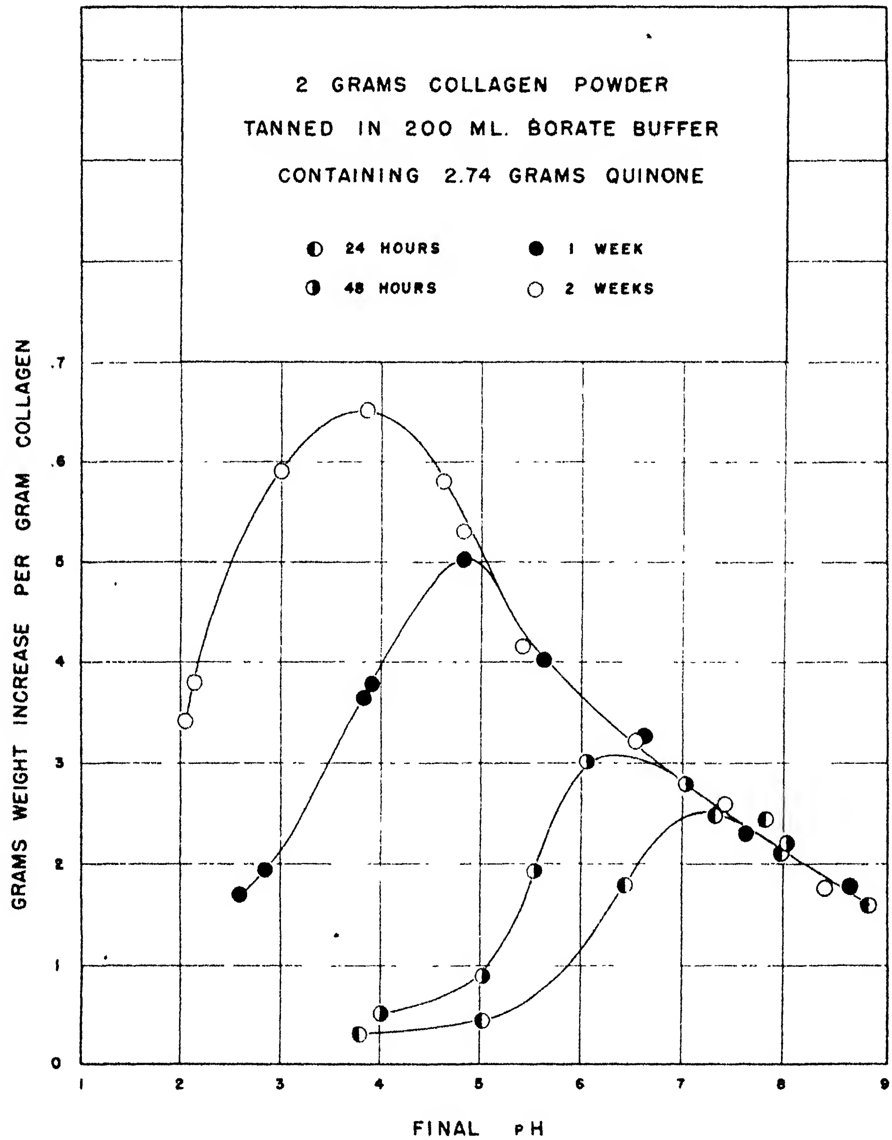


Figure 134

point at the identical pH value as before; therefore, they concluded that the minimum value was not in any way related to the isoelectric point of the collagen.

Stecker and Highberger next considered the possible effect of the nature

of the buffer system upon the quinone fixation. This investigation was carried out in a manner similar to that used in their earlier work, but in this

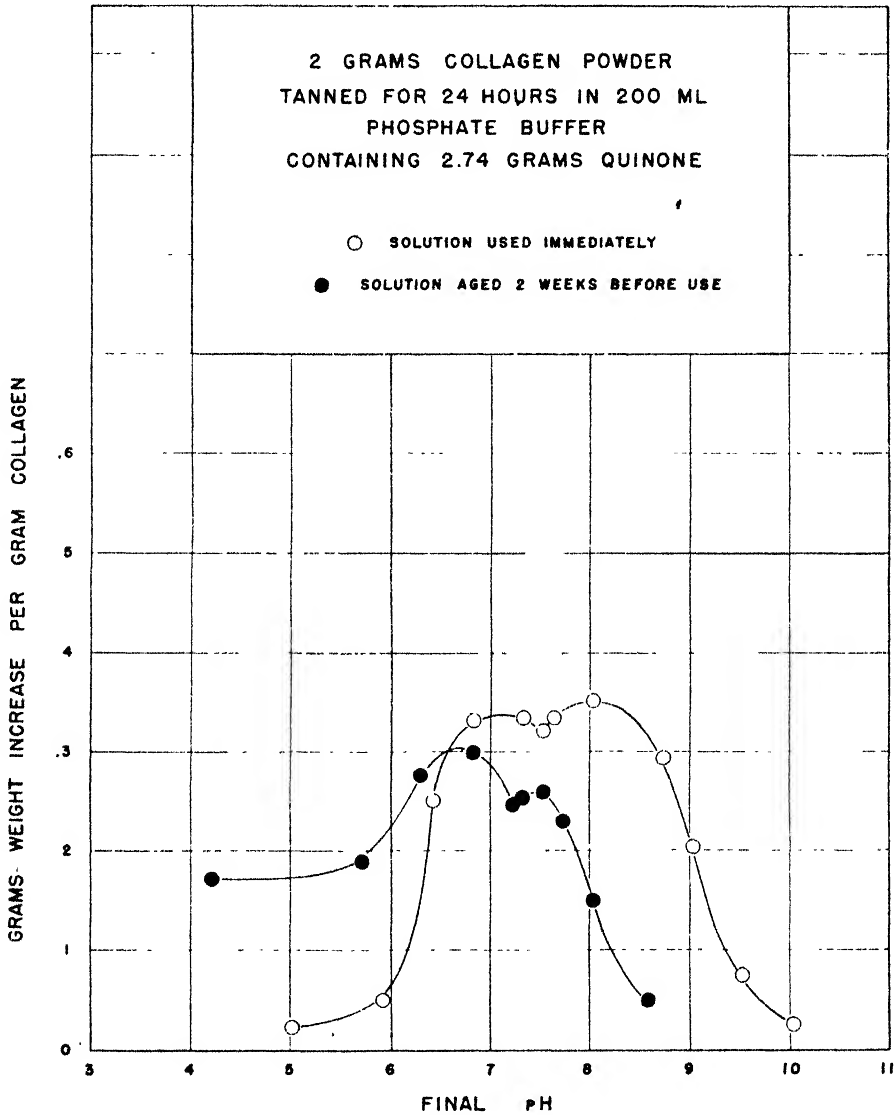


Figure 135

case boric acid-sodium tetraborate buffer mixtures were used. Their data for the borate buffer systems indicate the very significant influence of the nature of the buffer system upon quinone fixation, in that the alkaline maxi-

mum and the minimum point have completely disappeared. In their place, however, there appears a single maximum which gradually shifts toward a more acid pH value with increasing time of contact.

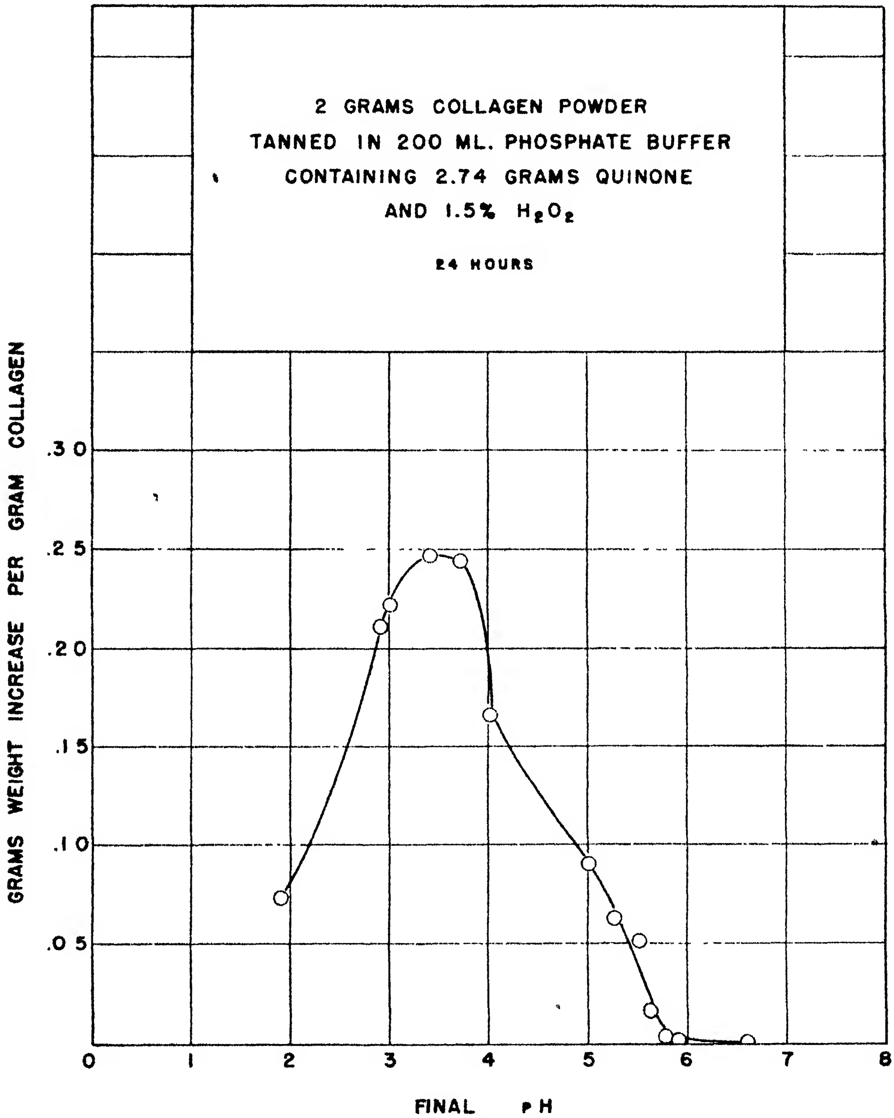


Figure 136

Stecker and Highberger believed that in the case of quinone fixation by collagen in the presence of phosphate, quinone is not fixed alone, but is

accompanied by other substances. They further postulate that these additional substances must be derived from quinone through reactions bringing about its oxidation and polymerization. For this reason, these investigators interpret their data, as shown by the figures as follows: fixation in the acid zone up to a pH value of 7.0 represents combination of monomeric quinone with collagen, and such fixation is favored by increasing pH value; in the pH range 7.0-7.5, the oxidation of the quinone increases so that it predominates over the monomeric combination reaction, and for this reason quinone fixation

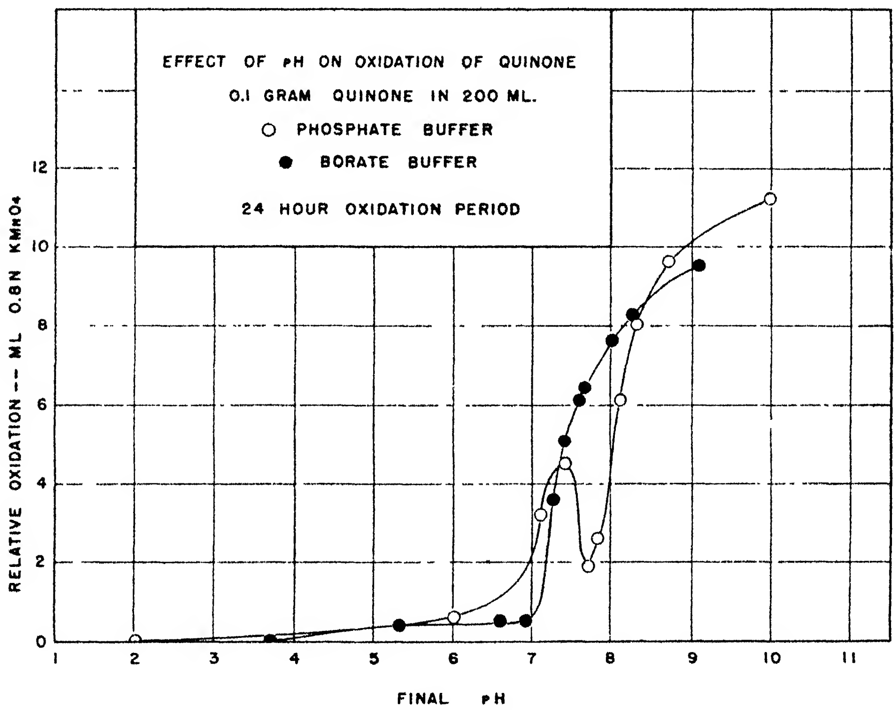


Figure 137

then decreases; at still higher pH values, the combination of collagen with the quinone polymer comes into play, and this effect obtains until pH 8.0 is reached; above this, the oxidation reaction is so predominant that monomeric fixation practically ceases. Stecker and Highberger assume that the polymer is formed over the entire pH range, but much more rapidly in the alkaline zone, and that it combines appreciably with collagen only above pH 7.5. They believe that the weight-gain values in the acid range, over long periods of time, are due to the slow formation and precipitation of this polymer within the

collagen structure itself. In this form, the polymer is not chemically bound by the collagen but is merely held mechanically within the structure.

Stecker and Highberger studied further the oxidation and polymerization of *p*-benzoquinone solutions. They point out that rapid oxidation is known to occur in even mildly alkaline solutions. Diels and Kassebart and Diels and Preiss have shown quinone to be quite capable of polymerization. *X*-ray and microscopic examination have demonstrated that a polymer of quinone may be deposited within the structure of quinone-treated collagen.

In their study of the pre-aging of quinone solutions for some two weeks, Stecker and Highberger found a shift of both the maximum and minimum fixation points to a lower pH value, and that the maximum fixation value on the alkaline side had markedly decreased. They also noted that the weight gain at pH values less than 6.5 had increased. They interpreted these facts as having resulted from the slow building up of the polymer, in the acid zone, during the aging period. To substantiate their interpretations further, Stecker and Highberger studied the effect of accelerated oxidation on quinone fixation. The accelerated oxidation was produced by adding 1.5 per cent of hydrogen peroxide to the *p*-benzoquinone solutions buffered with phosphate. Figure 136 shows their data for a 24-hour tanning period. These indicate definitely that accelerated oxidation causes the disappearance of the alkaline maximum fixation and the shift to a more acid zone of the acid maximum From such data, they postulate that at least part of the weight-gain increase of the quinone-collagen compound in the acid zone is due to the deposition of various oxidation products.

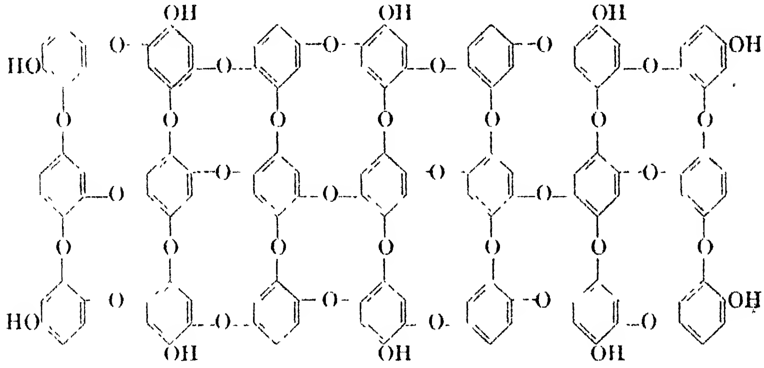
Stecker and Highberger next turned their attention to the oxidation of quinone in borate- and phosphate-buffered solutions. In this case the oxidation of the quinone solutions without collagen was investigated. Their investigation covered the pH range from 2.0 to 10.0 for a 24-hour period. The extent of oxidation was ascertained by a modified "oxygen required" determination. The determination was carried out by boiling an aliquot of the quinone solution with dilute sulfuric acid and a known excess of standard potassium permanganate for 10 minutes. A known excess of standard oxalic acid was then added and the solution was then back-titrated with standard permanganate solution. In this way, the amount of permanganate used in the oxidation of the quinone was estimated. Their data are shown in Figure 137. They indicate that but little oxidation occurs in the borate system below pH 7.0, but that above this there is a sharp increase in oxidation. With the phosphate system, appreciable oxidation occurs at pH 6.0, increasing to a maximum value at pH 7.4, decreasing to a minimum value at pH 7.7 and then increasing at a constant rate. Interpretation of these data indicates that the results for oxidation of quinone solutions in the absence of collagen are in good agreement with their previous work dealing with the phosphate-quinone collagen

system, and that the inflection in the curves may be ascribed to the formation of a polymer which is more resistant to oxidation than is *p*-benzoquinone.

Highberger points out in a recent personal communication that his attention was first directed to the possibility of polymerization by his x-ray studies, in which it was shown that quinone was the only tanning agent out of line with the general rule that only large molecules alter the x-ray diagram of collagen on tanning.

Stecker, in further unpublished work dealing with the nature of the quinone polymer, showed that the molecular weight, as determined by the Signer isothermic distillation method, was dependent on temperature, decreasing with increasing temperature. The minimum molecular weight of 2280 was obtained at 43° and remained constant up to 55°. These determinations were made in acetone solution and might possibly be higher in aqueous solutions.

Stecker points out that the polymer has definite acidic properties and proposes a tentative structure, based on molecular weight and hydroxyl group determinations. This structure is shown below:



Stecker found the hydroxyl content of the polymer to be 4.5-5.0 per cent, and thus he rules out the various chain structures, which might be postulated, since these would have a much higher hydroxyl content.

Highberger and Kersten[1] in 1938 examined various quinone tannages by means of x-ray spectrographic analysis. The only previous work dealing with the collagen-quinone compound was performed by Katz, in which he showed an x-ray diagram of tendon collagen treated with quinone. Highberger and Kersten treated collagen powder with quinone in phosphate buffers for varying periods. The tanned collagen was washed and dried. The weight gain was determined and portions of the treated collagen powder were used for x-ray analysis. Plates 12 and 13 show the x-ray pattern for several collagen-quinone compounds. Plate 12 shows a reproduction of a sector photograph in which the original collagen diagram is compared with those produced by

collagen treated for 6 hours and for 2 weeks. These investigators state that the pronounced blurring of the interference due to the side chain spacing is very similar to that which occurs on tanning with vegetable tanning liquors, as is the loss of intensity of the 2.8 Å ring.

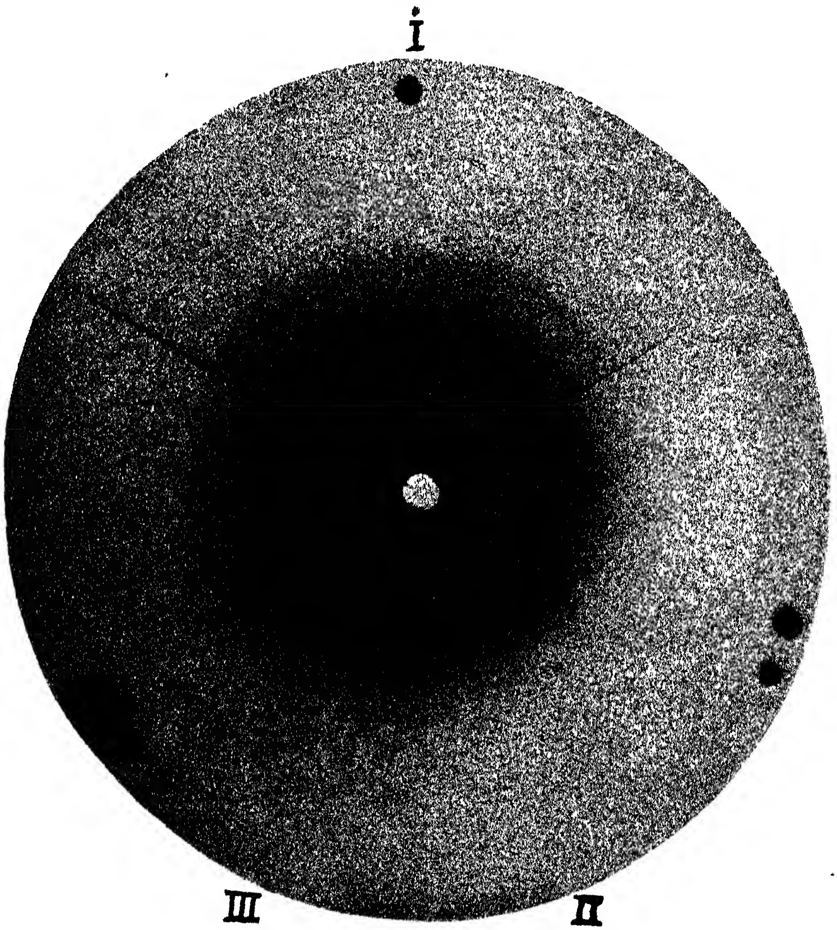


Plate 12

I. Original Collagen.
II. Tanned 6 Hours in Quinone at pH 8.03. Contains 0.189 Gram Qunione per Gram Dry Collagen.
III. Tanned 2 Weeks in Quinone at pH 8.03. Contains 0.357 Gram Quinone per Gram Dry Collagen.

Highberger and Kersten in making their first studies did so without first determining the bound quinone, and found as a consequence, for the 4-week

tanning period at pH 10.0, that their x-ray diagram showed no alteration of the original collagen diagram. Since Thomas and Kelly had shown a maximum and quite large fixation of quinone under such conditions, it appeared as though quinone binding produced little or no alteration in the collagen pattern.

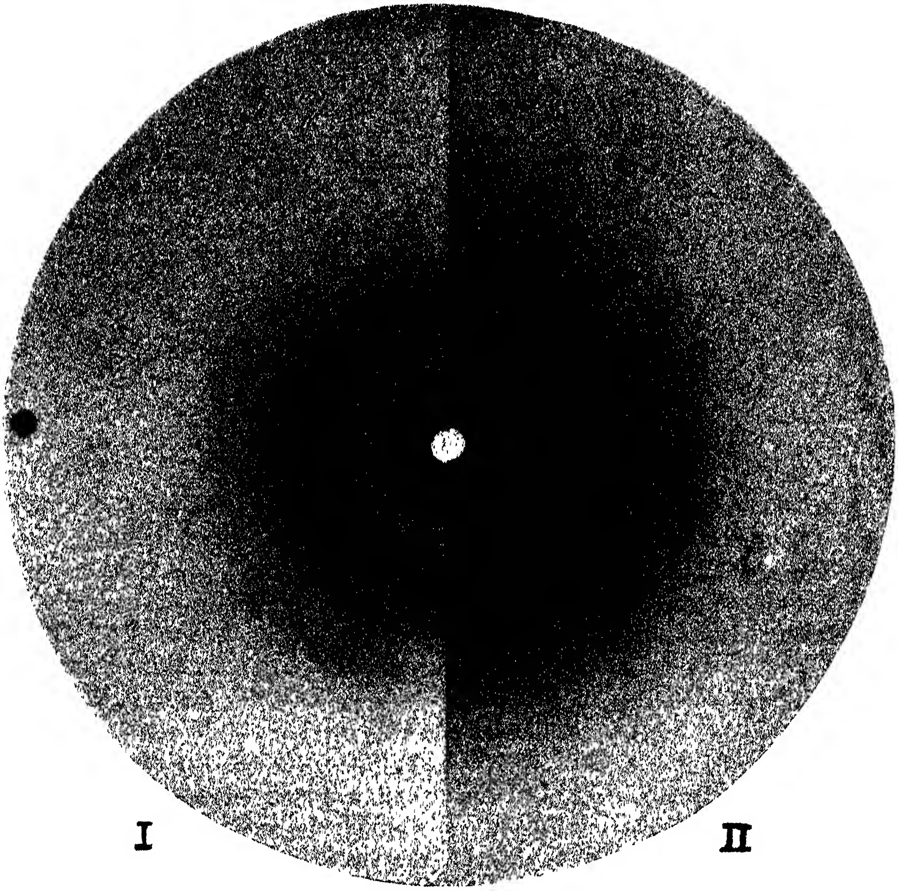


Plate 13

I. Collagen Tanned 6 Hours in Quinone at pH 9.97. Contains 0.207 Gram Quinone pe Gram Dry Collagen.
II. Collagen Tanned 15 Days in Quinone at pH 9.97 Contains 0.149 Gram Quinone per Gram Dry Collagen.

Under exact control of hydrogen ion and other experimental conditions, Highberger and Kersten found that there is an initially rapid tannage (pH 9.97) followed by an automatic and gradual detanning action. Such data are given in Table 166.

Table 166. Fixation of Quinone by Collagen Powder Grams Quinone Fixed per Gram Dry Collagen.
(By Weight Increase)

pH	6 Hours	24 Hours	15 Days
5.94	0.048	0.078	0.212
8.02	0.157	0.163	0.217
9.97	0.207	0.176	0.149

The results of Thomas and Kelly were in error in this regard, since they did not actually work at the particular pH value shown in their data. As at that time there existed no method for accurately determining quinone pH values, they used phosphate buffers of known pH values, assuming that the quinone solutions would not greatly change the hydrogen ion concentration. In the alkaline zone, this supposition is untrue, and the effect of quinone upon the pH value of the buffers can be quite large, as shown by Highberger and Kersten in Table 167.

Table 167

pH before adding quinone	pH after adding quinone
5.94	5.97
7.87	7.75
9.91	8.03

Highberger and Kersten state: "It thus appears that the samples which had been tanned at pH 10.0 for 4 weeks probably contained very little quinone, and consequently showed an unaltered collagen diagram. This is confirmed by the results shown in Plate 13, which is a reproduction of a sector photograph comparing the patterns yielded by the samples tanned at pH 9.97 for 6 hours and for 15 days respectively, the quinone fixation data for which are given in Table 166. The fact that the interferences in the diagram obtained from the 15-day sample are much more sharply defined than those in the pattern from the 6-hour sample shows that the smaller weight gains with increasing time cannot be ascribed merely to solution of collagen, but must be due to an actual removal of quinone. At present the most probable explanation of this effect appears to be that the fixation of quinone from the alkaline solutions is a reversible process, and that an equilibrium is established. Due to the high alkalinity of the solution, the free quinone is readily oxidized, and the equilibrium, being thus disturbed, is re-established by the dissociation of quinone from the quinone-collagen compound. Such a reaction might proceed until all of the quinone originally fixed had been removed."

These investigators further state that since quinone is a definitely crystalline substance, superposition of its diagram upon that of collagen would not be expected to give the type of result obtained, which is similar to that obtained with vegetable and chrome tannages. Relative to their studies, Highberger and Kersten further comment: "The characteristic effects which

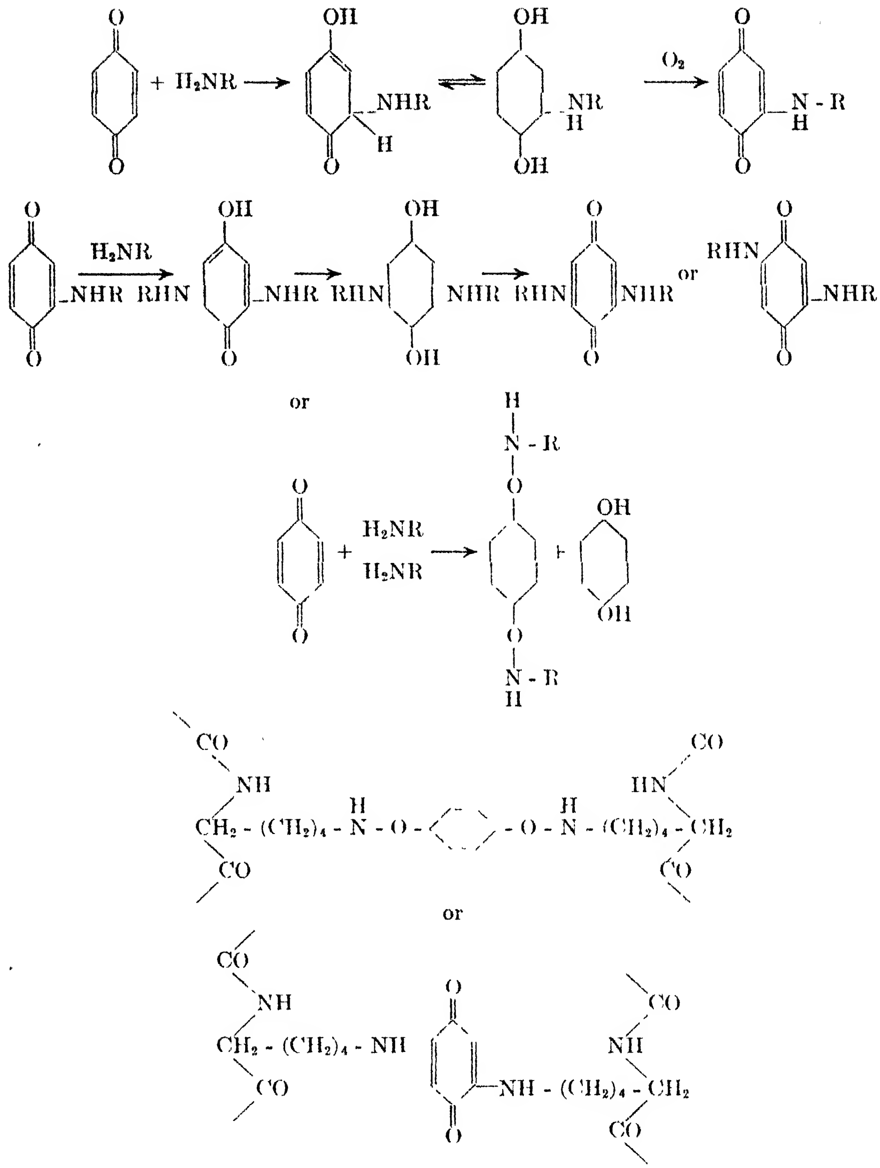
we have found are similar to those classed by x-ray spectrographers as 'line broadening,' although much more pronounced than those usually encountered in the case of inorganic crystalline materials. Such line broadening effects are generally the result of one of two causes. These are, first, particle size, and second, variations of the crystal lattice due to mechanical or other strains. In the present case the effect of particle size must evidently be ruled out, and it is our opinion that the effects noted are due to an actual distortion of the fiber structure (or semi-crystalline lattice) of the collagen, due to the introduction of the tanning agent. This is supported by other evidence, not yet published, among which may be mentioned the fact that it is possible to obtain similar effects, and to enhance those already obtained in tanning, by mechanically distorting the collagen fiber.

"This view is in agreement with that expressed by Jordan Lloyd, who attributed the loss of definition of the side chain interferences to the fixation of tanning agent at the strongly polar side chains, and that of the 2.8 Å interference to a marked disturbance of structure. Our own opinion at the present time is that the structural disturbance due to tanning is the resultant of two forces operating at right angles. The first of these is due to the disturbance of the balanced electrical forces at the polar side chains, which results in a lateral distortion of the structure. Possibly as a result of this, and possibly from the actual saturation of the weaker attractive forces along the back-bone, a longitudinal force is set up which produces a condition of 'slip' along the fiber axis. The latter condition results in the weakening and disappearance of the 2.8 Å interference. Such an idea readily explains the loss of strength noted by Jordan-Lloyd[2] where this interference was found to be weak or absent."

In 1912, Theis and Blum[5] made a preliminary study of the mechanism of the collagen-quinone reaction. They pointed out that there was a tendency to regard the phenolquinones and hydroquinones as loose molecular compounds whose structure cannot be numerically expressed by changes in valence.

Quinone has at least two reactive groups and therefore should be capable of reacting with at least two active amino groups of the polypeptide chain. From a theoretical standpoint, we might view the reaction of quinone with an amine somewhat as shown on page 404.

In their preliminary investigation, Theis and Blum studied the change in shrinkage temperature of goat skin tanned for 48 hours at 20° in a one per cent solution of quinone. The pH range employed varied from 1.0 to 10.0. After 48 hours, the excess quinone solution was removed from the tanned leather by means of pressure and the shrinkage temperature of each sample determined. Figure 138, curve A, shows the shrinkage temperature for each pH value. The trend of this curve demonstrates nicely that actual tanning



takes place over the pH range 2.5 to 10.0, reaching a maximum value, as regards shrinkage temperature, at pH 6.0. The shrinkage temperature in the range pH 4.0 to 7.0 is well above that of normal formaldehyde tannage and at pH 6.0 considerably above that of vegetable tannage. The trend of the quinone fixation-pH curve is similar to that for other anionic tannages. Curve

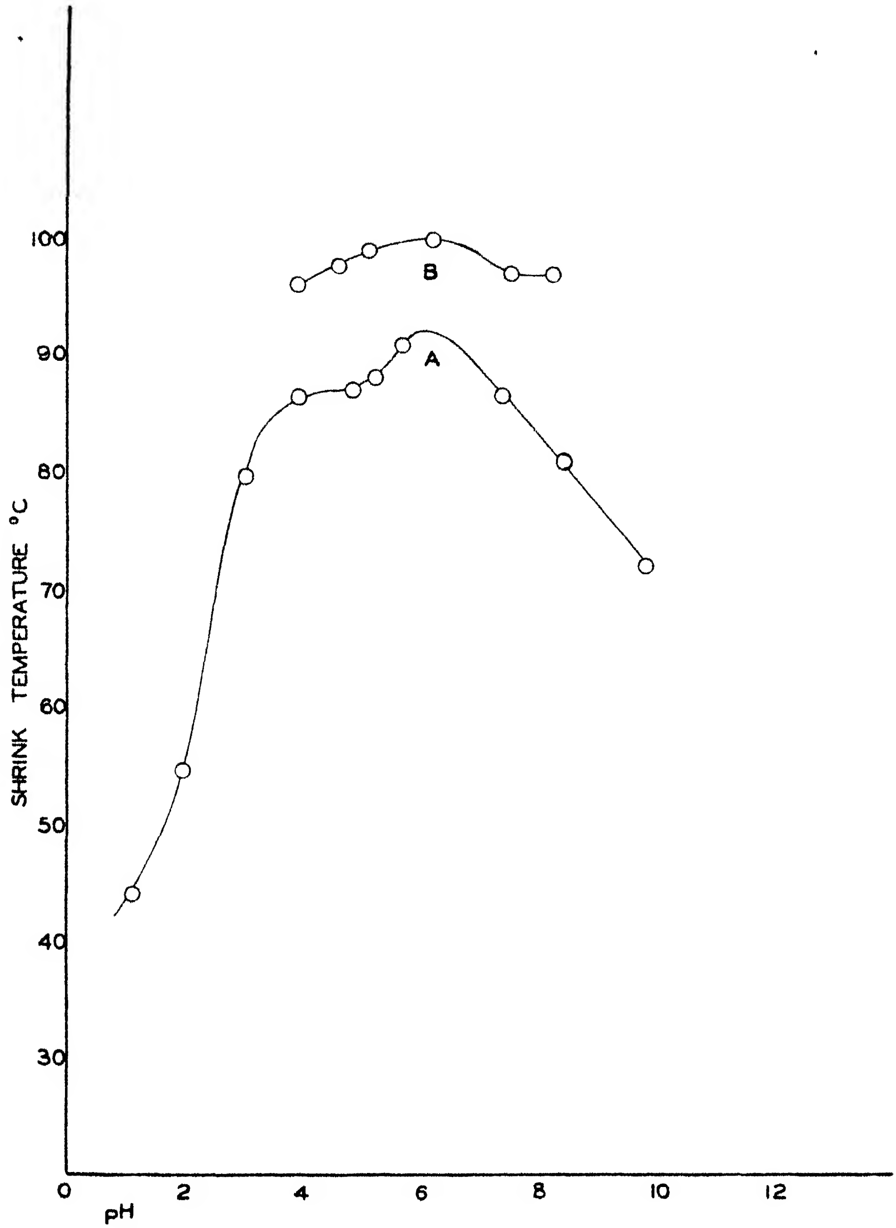


Figure 138. Shrinkage temperature of quinone-tanned leather.

B of this same figure shows the increase in shrinkage temperature taking place upon retannage of the quinone leather with formaldehyde. The increase in shrinkage temperature indicates that even though quinone reacts with available amino groups of the protein chain, not all of these groups react with

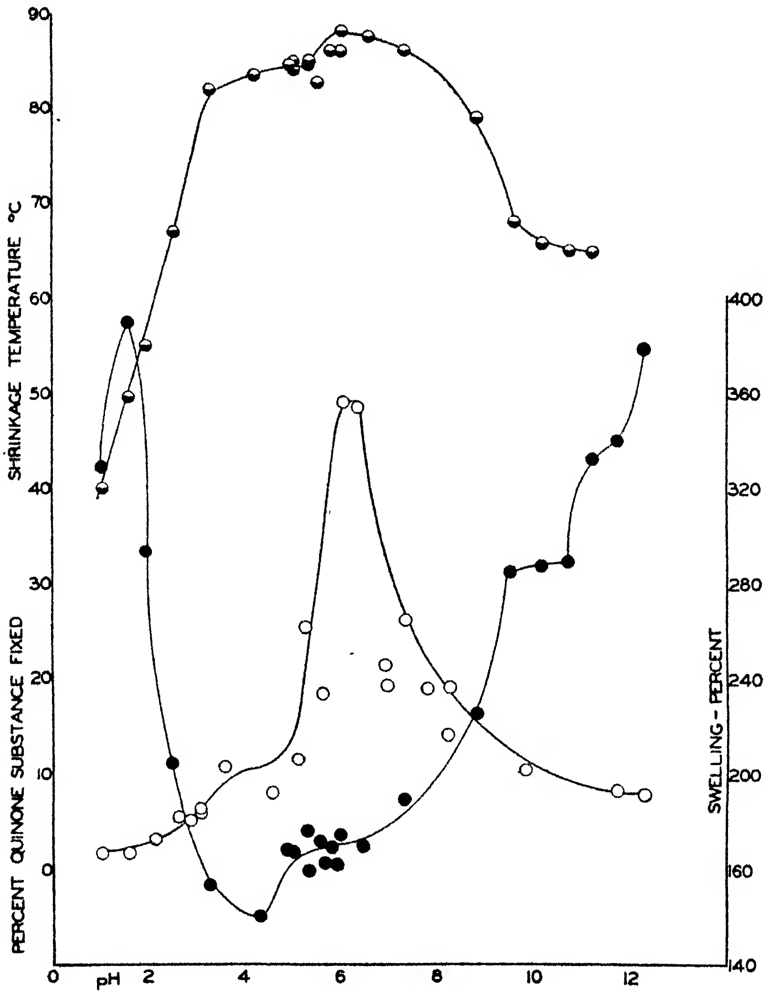


Figure 139

the quinone; and that in all probability additional amino groups react with the formaldehyde to form a greater number of interchain bonds, thus increasing the structural stability and shrinkage temperature of the collagen.

In subsequent studies, Theis and Lams[6] investigated the effect of hydrogen

ion concentration upon quinone fixation, upon swelling of the collagen-quinone substance, and upon the resulting shrinkage temperature of the quinone leather. For this work, they used properly prepared goat skin, a one per cent solution of quinone adjusted to the particular pH value noted, and a tanning period of 96 hours. After tanning the skin was removed, pressed at 10,000 pounds per square inch, dried at 105° over night, and the increase in weight noted. This increase was taken as quinone bound. Additional pieces were tanned for determination of shrinkage temperature. The data so obtained are shown graphically in Figure 139. These data give an interesting picture of the collagen-quinone reaction and the following trends are indicated: (a) in the pH range 3.2 to 8.7 the shrinkage temperature of the leather is greater than 80°, reaching a maximum value of 88° at pH 6.0; at pH values lower than 3.2 or higher than 8.7, tanning action as measured by shrinkage temperature decreases markedly; (b) a maximum weight gain or maximum quinone fixation occurs at pH 6.0 and sharp decreases in this factor take place on either side of this pH value; (c) maximum quinone fixation occurs in the zone of minimum swelling.

The trend of the weight-gain curve corresponds in general to that obtained by Stecker and Highberger for their borate buffer systems. These data are particularly interesting for comparison since they represent values for unbuffered systems and a 96-hour tanning period. Theis and Lams[6] found that for the unbuffered solutions, the maximum quinone fixation may vary from pH 6.0 to pH 8.0.

The swelling and plumping taking place during quinone tannage are of particular interest since they picture the maximum quinone fixation and maximum shrinkage temperature as occurring in the zone of minimum swelling. The swelling curve shows a definite maximum at pH 1.6, a first definite minimum at pH 4.4, a plateau in the pH range 5.0 to 6.0 with an indication of a second minimum value at pH 6.5, and finally an alkaline swelling at pH values greater than 7.0.

The shrinkage temperatures of the various quinone tannages are of interest since the maximum value occurs at pH 6.0 and is well in line with similar values obtained for other anionic tannages, such as vegetable and anionic chrome tannage. Further, the occurrence of maximum shrinkage temperature in the minimum swelling zone again substantiates the postulation of Theis *et al.* relative to the explanation of shrinkage temperatures and degree of leathering.

In Figure 140 the acid- and base-binding capacity of quinone tanned leather are shown. These curves were obtained in a manner similar to others described in Chapter 4. For this investigation Theis and Lams placed properly prepared collagen in a one per cent quinone solution, made 0.1N with respect to potassium chloride, and then adjusted such solutions to the

desired pH value with either hydrochloric acid or potassium hydroxide. The period of reaction was 96 hours. The tanned collagen strips were then removed, pressed dry, air-dried and ground to a fine powder. The collagen powder was then analyzed for protein, acid or base bound, chloride bound, and oxidizing value.

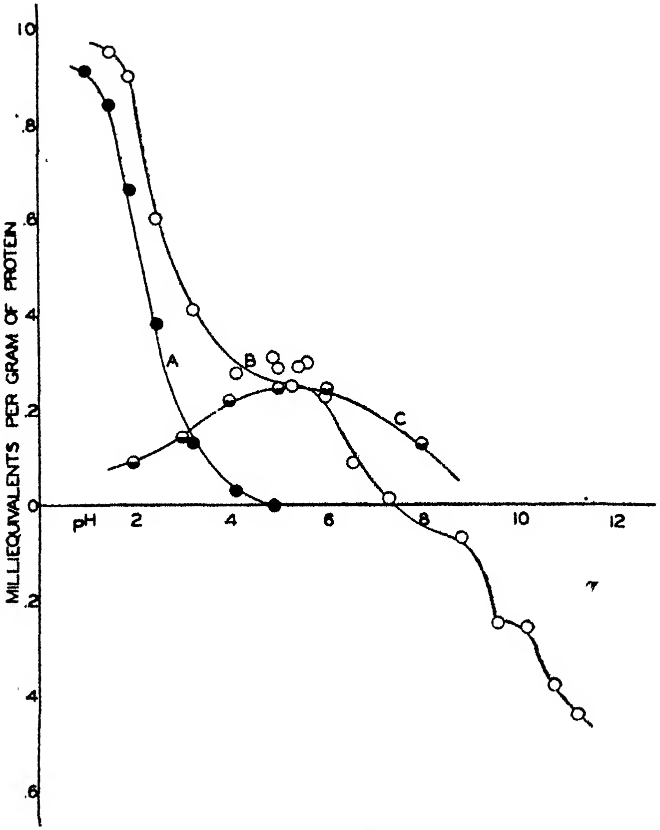
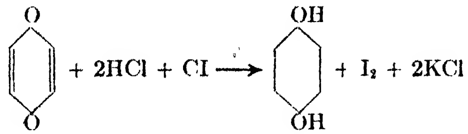


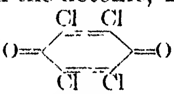
Figure 140

Curve B of Figure 140 shows the total acidic substance bound over the pH range studied, and curve A shows the HCl bound in the acid zone. The difference between curves A and B represents the acidic substances of the quinone bound by the collagen. Stecker has already called attention to the acidic properties of the quinone polymer, and it may very well be that it is this acidic polymer held within the skin structure which is binding with the appropriate basic groups of the collagen.

That quinone is bound in a manner other than through carbonyl linkage is shown by curve C, which represents the residual oxidizing power of the quinone-collagen substance. It is well known that quinone in the presence of HCl will liberate iodine quantitatively as shown:



If the quinone should bind with collagen in a manner such that some of the ketonic groups are still available, as postulated by Theis and Blum, then the collagen-quinone compound should show some oxidizing ability similar to that exhibited by *p*-benzoquinone. That this is to some extent true is shown by curve C of the Figure 140. The substance bound by the collagen exerts a definite oxidizing effect; this reaches a maximum value in the pH zone 5.0-6.0, and decreases on either side of this point.

Since they had postulated a binding of quinone with collagen by means of groups other than the ketonic, Theis and Lams investigated the reaction of tetrachloroquinone  with collagen and found that this reagent possesses no tanning properties, since the collagen so treated had the same shrinkage temperature as untreated protein.

In subsequent work, Theis and Lams studied the reaction between quinhydrone and hydroquinone with collagen over a pH range of 1.0 to 12.5. In this investigation, a one per cent solution of the reagent was used and the time of treatment was 96 hours at a constant temperature of 20°. These investigators found, as might be expected, that quinhydrone exhibited tanning properties similar to those of quinone, in that the shrinkage temperature of the quinhydrone-collagen compound increased to a maximum value at about pH 6.0. The hydroquinone-collagen compound, on the other hand, showed little increase in shrinkage temperature at any hydrogen ion concentration; hence the supposition that hydroquinone exerts little or no tanning action. The data taken are shown in Table 168.

There is little doubt that quinone is an active tanning agent, and it can become an important one in the future. Under proper conditions of time, temperature and concentration, leather so produced can be made to stand boiling water.

The exact mechanism of the reaction between collagen and *p*-benzoquinone remains essentially somewhat in doubt, but it may in reality be a combination of some three different reactions: (1) a reaction of the ketonic groups of the quinone with amino groups of the collagen; (2) a reaction of the 2-5 and 3-6

Table 168

Quinhydrone		Hydroquinone	
Tanning pH	Shrinkage Temp (° C)	Tanning pH	Shrinkage Temp (° C)
1.0	37	1.1	39
1.5	40	1.5	40
2.1	44	2.1	41
3.0	49	3.1	44
3.4	65	3.7	47
4.4	78	4.6	49
4.8	79	5.6	52
5.3	80	6.4	60
5.9	80	6.9	65
6.5	80	7.4	68
7.1	76	8.7	67
7.9	64	9.6	67
8.2	56	10.8	64
9.2	56	11.6	63
10.5	58	12.3	61
11.0	56		
11.7	55		
12.2	55		

positions of the quinone with the free amino groups of the collagen; and (3) a reaction of the acidic hydroxyl groups of hydroquinone and quinone polymer with a number of the basic groups of the collagen.

It appears to the authors that much remains to be investigated before we can obtain a clear picture of the true reaction. Analogy can be drawn between quinone and vegetable tanning reactions, since they all are essentially anionic in character and react with collagen, binding both tanning and non-tanning substances. In the two cases, retannage with formaldehyde increases the degree of tannage as measured by the shrinkage temperature and thus indicates that certain specific groups are available for combination with other tanning compounds.

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Chapter 14

Chemistry of Chromium Salts

Our present knowledge and understanding of the chemistry of the salts of chromium, particularly of those which are used in tanning, are based upon the concepts of Alfred Werner. This fruitful approach was introduced into leather chemistry in 1923 by Stiasny and by Gustavson. The description and discussion of chrome compounds in Stiasny's great book "Gerbereichemie"[1] is probably the best and most complete treatise to be found; and we refer those readers who are particularly interested in the subject of general chrome chemistry to this book. In this chapter we shall give only a brief description of those chrome compounds which are of particular interest in our understanding of chrome tanning.

In 1920, Werner[4] summarized his general theory as follows:

"Even when, to judge by the valence number, the combining power of certain atoms is exhausted, they still possess in most cases the power of participating further in the construction of complex molecules with the formation of very definite atomic linkages. The possibility of this action is to be traced back to the fact that, besides the affinity bonds designated as principal valencies, still other bonds on the atoms, called auxiliary valencies, may be called into action."

In other words, Werner assumes that even though the primary valence-combining capacity of an atom may be exhausted, some atoms can still combine with other atoms, radicals, or molecules by means of secondary valence and thus build up more complex molecules. The number of such groups which an atom can hold by means of secondary valence is termed its *coordination number.* The central atom together with its coordinated groups, usually referred to as a *complex,* functions as a unit, as we shall see. The value of the coordination number varies from two to twelve; in most cases, as with chromium, it is six. This value is a function of *available space* around the central atom.

According to Werner,[4] chromium salts may be divided into seven types. These types may be illustrated by considering the case of the simple salt, chromic chloride. There are three different forms of chromic chloride. The first, or α form, has the formula $CrCl_3 \cdot 6H_2O$; the second, or β form, is $CrCl_3 \cdot 5H_2O$; and the third, or γ form, is $CrCl_3 \cdot 4H_2O$. The three forms show

puzzling differences; while all three chlorine atoms may be precipitated from the α salt by silver nitrate in aqueous solution, only two are precipitated from the β salt, and only one from the γ. When conductivity measurements are made of aqueous solutions of the three salts, it is found that they function as four-, three-, and two-ion solutions respectively. Werner's explanation of these differences is illustrated by the following structural formulae:

(1)

$$\left[\begin{array}{ccc} H_2O & & H_2O \\ H_2O & Cr & H_2O \\ H_2O & & H_2O \end{array}\right]^{+++} 3Cl^-$$

hexaquo-chromi chloride (α form)

(2)

$$\left[\begin{array}{ccc} H_2O & & Cl \\ H_2O & Cr & H_2O \\ H_2O & & H_2O \end{array}\right]^{++} 2Cl^-$$

chloro-pentaquo-chromi chloride (β form)

(3)

$$\left[\begin{array}{ccc} H_2O & & Cl \\ H_2O & Cr & Cl \\ H_2O & & H_2O \end{array}\right]^{+} Cl^-$$

dichloro-tetraquo-chromi chloride (γ form)

When the α salt is dissolved in water, it ionizes to yield its three chloride ions, whereas the chlorine atoms which have entered the coordination sphere of the β and γ salts are not ionizable. And when an ion, such as the chloride noted above, enters the coordination sphere, it carries its charge with it and thus reduces the net charge of the chromium complex, as the formulas show. The entrance of un-ionized groups into the complex will not, of course, affect its net charge.

We may now consider four more possible types of chromium chloride, whose formation may occur in the presence of hydrochloric acid and sodium chloride, whereby all complexly held water molecules may finally be displaced.

(4)

$$\left[\begin{array}{ccc} H_2O & & Cl \\ H_2O & Cr & Cl \\ H_2O & & Cl \end{array}\right]$$

trichloro-triaquo-chromium

(5)

$$\left[\begin{array}{ccc} H_2O & & Cl \\ H_2O & Cr & Cl \\ Cl & & Cl \end{array}\right]^{-} Na^+$$

sodium tetrachloro-diaquo-chromiate

(6)

$$\left[\begin{array}{ccc} H_2O & & Cl \\ Cl & Cr & Cl \\ Cl & & Cl \end{array}\right]^{--} 2Na^+$$

sodium pentachloro-aquo-chromiate

(7)

$$\left[\begin{array}{ccc} Cl & & Cl \\ Cl & Cr & Cl \\ Cl & & Cl \end{array}\right]^{---} 3Na^+$$

sodium hexachloro-chromiate

We thus note the important fact that solutions of chromium chloride may contain chromium nuclei with a great variety of electrical charge. This charge may vary from three positive charges, through neutrality, to three negative charges. The fact that chrome tanning liquors often show both cathodic and anodic migration is thus explained. Later we shall discuss the seven types of compounds described above in their relation to chrome tanning.

The reader will have noted the nomenclature employed in describing the compounds above. This nomenclature was devised by Werner to define the nature of compounds on the basis of his theory. The name of a cationic complex starts with the complexly held acid groups (chloro, sulphato, formato,

etc.) followed by other coordinated groups (aquo, hydroxo, etc.); then comes the metallic element (chromi, cobalti, etc.); these terms are then followed by the name of the acid radical which is outside the complex. This terminology is illustrated by the β salt noted above. A neutral, uncharged complex is given a name ending with that of its metallic element. Thus the compound shown in the fourth formula above is designated: trichloro-triaquo-chromium. When anionic complexes are considered, the name of such compounds begins with the cation outside the complex and terminates with the name of the central atom of the complex, this name ending with the suffix "ate"; see the seventh formula above. The valence of the central atom of the complex is indicated by its ending, thus: it is *a* for a valence of 1; *o* for 2; *i* for 3; *e* for 4; *an* for 5; *on* for 6; *in* for 7; and *en* for 8. The term "chromi" used in the formulas above indicates the trivalency of chromium.

Addition and Penetration Compounds

Werner distinguishes between addition and penetration compounds. When platinic chloride and potassium chloride combine: $PtCl_4 + 2KCl \rightarrow [PtCl_6]K_2$, we note that the four chlorine atoms originally combined with the platinum atom are present in the nucleus, along with the two chlorine atoms formerly combined with the potassium atom. This is termed an addition compound. But when we consider sodium hexachloro-chromiate (see formula above), we note that the condition described for addition compounds is not fulfilled in the hypothetical combination of chromic chloride and sodium chloride according to the following equation:

$$[CrCl_2(H_2O)_4]Cl + 3NaCl \longrightarrow [CrCl_6]Na3 + 4H_2O$$

In this reaction the four water groups originally bound to the central chromium atom have been displaced by four chlorine ions, and the compound formed is called a penetration compound.

Penetrating Power of Various Anions

The ability of various anions to penetrate the complex and thereby displace water molecules is of importance in determining the tanning behavior of a chrome compound. Stiasny[2] has arranged the anions in a series of increasing penetrating power: NO_3, Cl, SO_4, $HCOO$, CH_3COO, SO_3, and C_2O_4. The penetrating power of the hydroxyl ion is evidently of approximately the order of the oxalate or tartrate ion. The penetrating power of the nitrate ion is very small, since chromium nitrates, or the basic chromium nitrates, always contain the nitrate radical in the ionized form, as, for example, $[Cr(H_2O)_6](NO_3)_3$. The penetrating power of the oxalate radical, on the other hand, is very great, and the oxalate radical is always complexly held in chromium oxalates. Anions may also displace other ions in the complex and follow, in this respect, the

same general order listed by Stiasny above. Thus the oxalate or tartrate radicals have great power of displacing complexly held sulfate ion; this is the reason for their ability to strip basic chrome sulfate-tanned leather of its fixed chrome. The analytical method devised by Thomas,[3] whereby complexly held sulfate groups in chrome leather are displaced by phosphate ions, is another example of the displacement of one group by another. On the other hand, the chloride ion with weak penetrating power is less able to displace complexly held sulfate groups.

We have dealt in the above paragraph with anions. Water molecules may also sometimes penetrate the complex; their ability to do so is not marked and is probably of the order of the nitrate and chloride. Water molecules may also displace complexly held ammonia groups from compounds such as hexammine-chromi chloride $[Cr(NH_3)_6]Cl_3$, forming a series of compounds with a decreasing number of complexly held ammonia groups until, finally, hexaquo-chromi chloride $[Cr(H_2O)_6]Cl_3$ is obtained.

Hydrolysis of Chromium Compounds

The behavior of aqueous solutions of chrome salts is determined largely by the hydrolytic changes which have occurred and by the secondary changes subsequent to hydrolysis. This subject is of importance in chrome tanning.

Stiasny[1] has differentiated the views regarding the hydrolysis of chrome salts held by Arrhenius and by Werner and Pfeiffer. According to Arrhenius, the progressive hydrolysis of chromic chloride would be represented thus:

$$CrCl_3 + HOH \longrightarrow CrOHCl_2 + HCl;\ CrOHCl_2 + HOH \longrightarrow Cr(OH)_2Cl + 2HCl;$$
$$Cr(OH)_2Cl + HOH \longrightarrow Cr(OH)_3 + 3HCl.$$

Werner and Pfeiffer, on the other hand, regard the hydrolysis as that of water removal from the complexly bound water groups, as follows:

$$HOH \longrightarrow OH^-H^+$$

whereby the progressive hydrolysis of chromic chloride would be expressed

$$\left[Cr(OH_2)_6\right]^{+++} Cl_3^- + HOH \longrightarrow \left[\begin{matrix}Cr\ OH \\ (OH_2)_5\end{matrix}\right]^{++} Cl_2^- + HCl;$$
$$\left[\begin{matrix}Cr\ OH \\ (OH_2)_5\end{matrix}\right]^{++} Cl_2^- + HOH \longrightarrow \left[\begin{matrix}Cr(OH)_2 \\ (OH_2)_4\end{matrix}\right]^{+} Cl^- + HCl;$$
$$\left[\begin{matrix}Cr(OH)_2 \\ (OH_2)_4\end{matrix}\right]^{+} Cl^- + HOH \longrightarrow \left[\begin{matrix}Cr(OH)_3 \\ (OH_2)_3\end{matrix}\right] + HCl.$$

Both the above concepts show that the hydrolysis of chrome salts is always associated with acid formation. And Werner and Pfeiffer's explanation makes it clear that those chrome salts which contain no complexly held H_2O groups are generally not hydrolyzed and hence do not react acid in solution.

The hydrolysis noted above may, of course, be accelerated by either the addition of alkali to the chrome solution or the introduction of skin substance;

the latter will combine with the free acid of the solution until the acid-combining capacity of the skin has been satisfied; and this, in turn, is a function of the concentration of available acid of the solution. The hydrolysis may, on the other hand, be retarded by the addition of acid to the chrome solution.

Basic Chrome Compounds

It will have been noted that when cationic chrome salts which contain complexly held aquo groups are hydrolyzed, a basic chrome salt (containing OH groups) and free acid are formed. This fact is of importance in chrome tanning, because, generally speaking, only those chrome compounds which are basic, or which become so in solution, have tanning power. Stiasny[1] points out that the hydroxyl groups of such basic compounds cannot be ionically contained, since, if this were true, their solutions would have to react alkaline, whereas they actually react acid. This inevitably leads to the conclusion that the hydroxyl groups are complexly bound. Stiasny[1] differentiates the two types of basic compounds to be considered: (a) a chrome complex which is either positively charged or is neutral, and (b) a chrome complex which is negatively charged. Examples of the two types are shown as follows, where A stands for, in this case, aquo groups and X any acid anion:

(a)

1/3 Basic compounds,

$$\left[\begin{array}{ll} Cr & OH \\ & A_5 \end{array}\right]^{++} X_2 \qquad \left[\begin{array}{ll} & OH \\ Cr & X \\ & A_4 \end{array}\right]^{+} X^- \qquad \left[\begin{array}{ll} & OH \\ Cr & X_2 \\ & A_3 \end{array}\right]$$

(a)

2/3 Basic compounds

$$\left[\begin{array}{ll} Cr & (OH)_2 \\ & A_4 \end{array}\right]^{+} X^- \qquad \left[\begin{array}{ll} & (OH)_2 \\ Cr & X \\ & A_3 \end{array}\right]$$

(b)

Monohydroxo compounds with anionic complex

$$\left[\begin{array}{ll} & OH \\ Cr & X_3 \\ & A_2 \end{array}\right]^{-} K^+ \qquad \left[\begin{array}{ll} & OH \\ Cr & X_4 \\ & A \end{array}\right]^{--} K_2^+ \qquad \left[\begin{array}{ll} Cr & OH \\ & X_5 \end{array}\right]^{---} K_3^+$$

Dihydroxo compounds with anionic complex

$$\left[\begin{array}{ll} & (OH)_2 \\ Cr & X_2 \\ & A_2 \end{array}\right]^{-} K^+ \qquad \left[\begin{array}{ll} & (OH)_2 \\ Cr & X_3 \\ & A \end{array}\right]^{--} K_2^+ \qquad \left[\begin{array}{ll} Cr & (OH)_2 \\ & X_4 \end{array}\right]^{---} K_3^+$$

Trihydroxo compounds with anionic complex

$$\left[\begin{array}{ll} & (OH)_3 \\ Cr & X \\ & A_2 \end{array}\right]^{-} K^+ \qquad \left[\begin{array}{ll} & (OH)_3 \\ Cr & X_2 \\ & A \end{array}\right]^{--} K_2^+ \qquad \left[\begin{array}{ll} Cr & (OH)_3 \\ & X_3 \end{array}\right]^{---} K_3^+$$

A further distinction between the (a) and (b) compounds noted above is to be found in their reaction to alkali. Addition of alkali to the cationic, or positively charged, compound reduces its positive charge, thus:

$$[CrA_5OH]^{++} \longrightarrow [CrA_4(OH)_2]^{+} \longrightarrow [CrA_3(OH)_3]$$

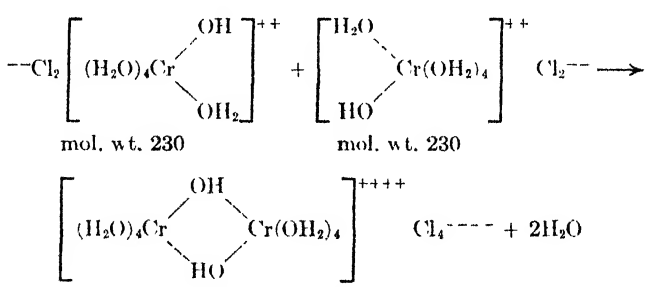
As these compounds approach the hydroxide state, their solubility decreases, and their tanning astringency increases.

When alkali is added to the anionic, or negatively charged compounds, their negative charge is increased, their solubility raised, and their tanning astringency is usually lowered. The course of this reaction may be expressed:

$$[CrA_2X_3(OH)]^{-} \longrightarrow [CrAX_3(OH)_2]^{--} \longrightarrow [CrX_3(OH)_3]^{---}$$

Olation, Polymerization, and Oxolation

A chrome compound is said to be "olated" when one or more of its hydroxyl groups is held between two chromium atoms, whereby it is attached to the primary valence of one chromium atom and to the secondary valence of the second chromium atom, thus: Cr—OH---Cr. The hydroxyl group or groups so held are not easily titratable with acid, whereas unolated hydroxyl groups are. And as will be noted, the process of olation leads to increased molecular size. Our knowledge of the phenomenon of olation in respect to its probably great significance in chrome tanning is largely due to the work of Stiasny[2] and his collaborators. They have shown that olation is favored by heat, increasing concentration, increasing basicity, and by time. When, for example, a 33⅓ per cent basic chromium chloride solution is heated, or is allowed to stand, the following changes are presumed to occur: two moles of the original compound combine to form a new compound, with the elimination of water, thus:



mol. wt. 230 mol. wt. 230

octahydrate-diol-dichrom-tetrachloride
mol. wt. 424

Stiasny points out that when higher-basicity compounds are employed, such as the 66⅔ per cent basic chrome chloride, the process of olation is extended, and greatly increased molecular size is attained. Thus six moles of the 66⅔ per cent basic chrome chloride may combine to form a compound containing six chromium atoms, having all twelve of its hydroxyl groups olated and having a molecular weight of 732. In the same way, six moles of the 83⅓ per cent basic salt may combine to form a 100 per cent olated compound with molecular weight of 1350. It should be noted that as the molecular size of these olated compounds increases, they approach a semi-colloidal degree of dispersion. This does not mean, of course, that *degree* of olation alone necessarily determines molecular size, for we have seen that the tetrachloride

shown above is completely olated but has a molecular weight of only 424, whereas the other completely olated compounds described have much greater molecular weights. Again, it will be noted that we may have a trinuclear $66\frac{2}{3}$ per cent basic chrome chloride complex which is 100 per cent olated and has a molecular weight of 366, whereas a hexanuclear complex which is only 50 per cent olated has a molecular weight of 840.

Stiasny[2] also points out that the process of olation results in an increase of acidity of a basic chrome solution, through the shifting of the hydrolysis equilibrium and the formation of free acid. This is suggested as one of the reasons a basic chrome liquor increases in free acidity upon standing; this is in addition to the effects of simple hydrolysis, already described.

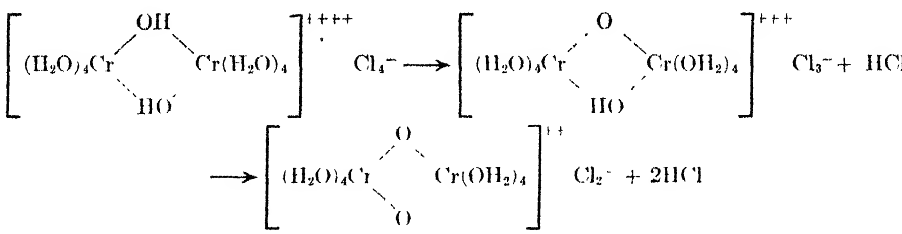
For the sake of simplicity of description we have employed the chloride compounds in illustrating the phenomena described above. But the same rules apply for chrome sulfate or other chrome compounds, taking into account, of course, the valency of the anion under consideration. The olation phenomenon just described may be reversed. Whereas the olated hydroxyl groups are relatively inert to added acid, they are not entirely so. Thus if we lengthen the time of contact with the acid, or if the concentration of the added acid is greatly increased, especially if heat is applied, de-olation will occur. De-olation is also favored by dilution of the chrome liquor. When de-olation takes place, the secondary valence bond between hydroxyl and chromium is loosened, and the chromium then satisfies its coordination number with a water molecule. Thus the octahydrate-diol-dichrom-tetrachloride described above would be resolved into its original two moles of $33\frac{1}{3}$ per cent basic chrome chloride; these are, in turn, easily acted upon by acid.

Stiasny[2] points out that the resistance of the hydroxyl olation linkage to the action of acids is greatly influenced by the nature of the other groups present in the complex. This is because some anions, such as the oxalate, possess great affinity for the central chromium atom, leaving but slight secondary valence force for maintaining the olation linkage of the hydroxyl group, or groups; consequently this linkage is easily broken. On the other hand, if the complexly held anion should be the chloride, which possesses much less attraction for the chromium atom, the secondary valence forces available for the olation bond are much greater, and thus this bond will show proportionately greater resistance to added acid. For these reasons Stiasny has arranged the anions in a series denoting their increasing effect of stabilizing the olation bond: oxalate, formate, sulfate, chloride, etc. It will be noted that this series is, as would be expected, the reverse of the series of increasing penetrating power given on page 413.

Increase of the molecular size of chromium compounds may also be brought about by simple polymerization processes, and these—like olation phenomena—are also influenced by increasing basicity, by time, and by tem-

perature. Polymerization differs from olation, however, in the fact that when the latter occurs water is eliminated.

Stiasny[2] also calls attention to the importance of oxolated chrome compounds which Werner has termed "oxo." This refers to compounds in which two chrome atoms are bound by an oxygen atom: Cr—O—Cr. These compounds are more stable and more resistant to acid than are olation compounds. They may be formed by continuous heating of olated compounds, with the liberation of acid, as follows:



The possible significance of these compounds in chrome tanning will be discussed later.

The introduction of Werner's concepts into both the theory and practice of chrome tanning has been largely responsible for much of the progress made in these fields in the past two decades. This will become apparent as we proceed with the discussion of their application. It must be pointed out, however, that in the case of certain hydrates—those containing an abnormally large proportion of water—we come to the least satisfactory application of Werner's theory. In the case of the alums, for example (including chrome alum), Werner believes their water is held in a polymerized form. Thus $KAl(SO_4)_2 \cdot 12H_2O$ would be expressed: $[Al(H_4O_2)_6](SO_4)_2K$.

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Chapter 15

Chrome Tanning

Prior to 1858 the great bulk of all leather was vegetable-tanned. In that year, however, Friedrich Knapp[39] made a discovery which was to bear rich fruit and which eventually led to great changes in the leather industry. Knapp discovered that leather could be made by treating animal skin with basic chromium compounds. Like many inventors, Knapp was unaware of the value of his discovery, nor was he able to solve the numerous technical and mechanical problems attendant upon the commercial production of chrome leather. In 1884, Augustus Schultz patented a chrome-tanning method known as the "two-bath" process; this is described below. Schultz was not a tanner but a dye salesman, and his interest in chrome tanning arose from the request of a customer for a method of tanning the leather used to cover corset steels which would be an improvement over the alum tannage then generally employed. In 1893, Martin Dennis secured a patent covering the use of a basic chrome compound as originally suggested by Knapp, in other words, a "one-bath" process. The successful practical application of chrome tanning was ultimately due to the efforts and ingenuity of several American tanners. In this connection the name of Robert Foerderer deserves special mention. Foerderer had not only great mechanical ingenuity but a scientific approach to the problem of chrome tanning as well. This may be judged from the following printed statement of his in 1899:

"The future of the great leather industry is dependent entirely upon skill and a knowledge of chemical scientific principles. Upon these depend the acceleration and cheapening of the tanning process. Our leather manufacturers must aim to be more than good machinists; they must be practical and thorough chemists."

Two-Bath Process

As has been noted, the first commercially applied chrome-tanning process was the two-bath method of Schultz. During the intervening years this method has been largely replaced by the one-bath process. This is because the latter is more easily controlled and, when properly applied, yields leather results which are fully as good and often superior to two-bath leather. This is probably the reason for the surprisingly small amount of available scientific

data on two-bath tanning. Today the process is employed mainly in the tanning of goat and kid skins, but even here the one-bath method is rapidly coming into favor. Two-bath tanning consists essentially of the following:

The usually bated skin, which may or may not be pickled, is agitated in a solution of sodium bichromate, sulfuric or hydrochloric acid, and sodium chloride. Chromic acid is formed in this solution and impregnates the skin, which becomes yellow in color. At this point the skin is but very slightly tanned, as judged by its heat stability. This is because the chromic acid must be reduced to the basic state before appreciable tanning occurs. Reduction is accomplished by placing the chromic acid-impregnated skin in a second bath containing a reducing agent, usually an acidified solution of sodium thiosulfate. The sulfurous acid formed reduces the chromic acid to the basic state, which compound combines with the skin and tans it. The tanned skin has a greenish-blue color.

The reaction in the first bath may be expressed as follows:

$$Na_2Cr_2O_7 + H_2SO_4 \longrightarrow Na_2SO_4 + H_2Cr_2O_7.$$

In 1912, Stiasny and Das[75] studied the reaction of the second bath and found that it may occur in several different ways, depending upon variations in reacting conditions as noted below.

(a) $H_2Cr_2O_7 + 2H_2SO_4 + 3Na_2S_2O_3 \longrightarrow 2CrOHSO_4 + 3Na_2SO_4 + 3S + 2H_2O$

(b) $4H_2Cr_2O_7 + 5H_2SO_4 + 3Na_2S_2O_3 \longrightarrow 8CrOHSO_4 + 3Na_2SO_4 + 5H_2O$

(c) $H_2Cr_2O_7 + 5H_2SO_4 + 6Na_2S_2O_3 \longrightarrow 2CrOHSO_4 + 3Na_2SO_4 + 3Na_2S_4O_6 + 5H_2O$

Stiasny points out that the reactions of these equations occur simultaneously and that the proportions of the individual reactions to the total reaction depends upon conditions: dilution, acid excess, and thiosulfate excess. It can be assumed that under the normal conditions of two-bath tanning, equation (a) constitutes from 30 to 40 per cent of the total reaction, (b) 10 to 20 per cent, and (c) 50 per cent. Referring to the sulfur precipitated in equation (a), he states that this is actually less in amount than the theoretical; this is because part of the sulfur is colloidally dissolved and also because it combines with the tetrathionate, (c), to form pentathionate: $Na_2S_4O_6 + S \rightarrow Na_2S_5O_6$. When sulfur is actually present in two-bath leather, however, it results only slightly from the reduction changes above, but results from the direct action of acid on thiosulfate: $Na_2S_2O_3 + H_2SO_4 \rightarrow Na_2SO_4 + SO_2 + S + H_2O$.

In 1924 Meunier and Chambard[61] reported the following observations: Skin leaving the first, or chromic-acid bath, shows no swelling; chromic-acid absorption by the skin increases with its concentration; the addition of either sodium chloride or sulfate to the first bath decreases the absorption of CrO_3 and increases the absorption of HCl or H_2SO_4; and chromic acid has some slight

tanning property, since the first bath causes partial dehydration of the skin. These observations have been confirmed by Innes.[36]

Theis and Kalb[85] have extensively studied the first bath of the two-bath process, and their results may be summarized as follows:

They employed three-inch squares of unhaired and bated goat skin which were dehydrated with acetone. Experimental methods were as follows: Except as noted, a volume ratio of three ml solution per each gram of hydrated prepared skin was maintained; time of treatment was 24 hours or more, and temperature was maintained at 21°. At the end of the first bath treatment the chromed skin was pressed at 3000 pounds per square inch. Part of the pressed skin was used for analysis, and the remainder entered the second bath. The second bath contained 20 per cent sodium thiosulfate and 10 per cent hydrochloric acid; the hydrated skin/solution ratio was one to three. After 24 hours' treatment, the tanned skin was removed and again pressed at 3000 pounds per square inch and analyzed. The hydrochloric acid used throughout was of 33 per cent strength.

Using the above methods, five variables of the first bath were studied:

(1) Variable concentration of potassium bichromate with added hydrochloric acid equal to one-half the bichromate concentration.

(2) Constant concentration of bichromate with variation of acid concentration.

(3) Constant concentration of both bichromate and acid with varying time of treatment.

(4) Constant total amount of bichromate and acid but varying volume ratio; in other words, variable concentration.

(5) Constant concentration of bichromate and acid; constant volume ratio but varying sodium chloride concentration.

The results of these experiments may be summarized as follows:

(1) When bichromate was varied between one and ten per cent with constant acid concentration, the final amount of chrome fixed increased up to six per cent bichromate given. Higher bichromate concentrations did not increase chrome fixation.

(2) When bichromate was maintained constant at six per cent and acid varied from one-half to six per cent, chromic-acid absorption decreased when three per cent of acid was exceeded.

(3) When six per cent bichromate and three per cent acid were employed and time of treatment varied up to 48 hours, it was found that chromic acid absorption reached its approximate maximum within one hour.

(4) When initial amounts of bichromate and acid given were kept constant but their concentration varied, the data indicated that the concentration of

the first bath had but little effect upon the amount of Cr_2O_3 fixed in the second bath treatment.

(5) When bichromate was kept constant at six per cent, acid at three per cent, and volume ratio three to one, and sodium chloride added in amounts varying from 0 to 15 per cent, the final fixation of Cr_2O_3 was decreased, as a function of salt concentration, and the shrinkage temperature of the final leather was lowered. Addition of sodium sulfate to the first bath also lowered the shrinkage temperature of the final leather. These results confirm the observations of Meunier and Chambard as to the action of adding salts to the first bath in respect to CrO_3 absorption.

Stiasny[76] has discussed the possible reasons for the alleged fundamental differences in the characteristics of leather produced by the two- and one-bath methods. It was long believed that the deposition of sulfur upon the leather fiber in the two-bath process tended to produce a softness of the leather, a characteristic feel, and a fine grain. But this contention is rendered doubtful by the fact that two-bath leather may be produced which contains but very little deposited sulfur, without exhibiting any detectable differences in characteristics, compared with that containing much more sulfur. Also, leather may be de-sulfured without undergoing noticeable change in characteristics. It is possible that the more uniform chrome fixation throughout the cross-section area of two-bath leather, compared with one-bath tannage, may be a factor. This more even distribution of fixed chrome in the case of the two-bath results from the fact that the chromic acid is evenly distributed throughout the skin prior to its reduction. In the case of one-bath tannage, combination occurs from the skin surfaces inward, and the finished leather usually contains more fixed chrome in its grain and flesh areas than in the center of the leather. But it must be remembered in this connection that, depending upon the method of pickling and tanning, it is quite possible to maintain a perfectly even distribution of fixed chrome throughout the cross-section area of one-bath leather. Stiasny states that the basicity of the fixed chrome compound is lower in two-bath than in one-bath leather. He believes that this, together with differences of olation degree and molecular size of the chrome compound in the two tannages, probably explains the difference in leather qualities. And we would suggest that the various anions formed in the two-bath process may greatly influence the composition and tanning behavior of the chrome compound, just as different anions affect one-bath leather.

Conflicting and unsupported statements are often heard to the effect that less fixed chrome is required to enable two-bath leather to stand the boiling test than for one-bath leather. It must be understood, in the first place, that very few of the best American chrome upper leathers remain unaffected in area when subjected to boiling water, whether of one- or two-bath tannage. The only significant and actual basis of comparison in this question would be

to tan pieces of the same skin by the two methods, fixing a wide range of Cr_2O_3, endeavoring to maintain the same leather basicity for both tannages, and then secure quantitative data of comparative percentage shrink of the leather as a function of temperature.

One-Bath Process

As its name implies, this method consists of tanning the hide or skin directly in one solution or "liquor," in contradistinction to the two-bath process just described. The main chrome compound present in one-bath liquors is usually the basic sulfate. Basic chrome chloride was once widely employed but has been generally replaced by the sulfate, which yields more satisfactory leather results. As we shall learn in considering glucose reduction of one-bath liquors, significant amounts of other chrome compounds may be present in such liquors.

The bated stock is first pickled, as described in Chapter 11. The pickle is usually a solution of sulfuric acid and sodium chloride, but the pickle composition is often varied in different tanneries and with different processes. Thus the acid used may be hydrochloric and salts other than sodium chloride may be employed, or may be added with the sodium chloride. The pickle may thus vary in composition, but the main object of the process is two-fold: to bring the skin into a uniform chemical and physical condition throughout its area on the one hand, and to prevent too rapid tannage (especially of the outer skin layers) on the other. The various anions present in the pickle solution may also markedly affect both the composition and the tanning behavior of the chrome liquor which is added.

The chrome liquor is sometimes added directly to the drum containing the pickled skins and the exhausted pickle liquor, and tannage then proceeds. Or, the pickling may be performed in a paddle vat and the pickled stock transferred to the drum for tanning. The tanning may also be performed in a paddle vat; this process usually involves weaker chrome liquors and longer tanning time than in the case of drum tannage. Another one-bath process employed to a limited extent is the so-called "dry tannage" This process consists of drumming the drained, bated but unpickled skin with a very small volume of highly concentrated chrome liquor. It is often assumed that this method means that the actual tanning is performed by very concentrated chrome liquors. This is usually not the case, however, if for no other reason than that such highly concentrated solutions are unable to diffuse adequately into the skin fiber. Tannage actually results because bated stock contains a large quantity of free water. This free water diffuses out of the skin and greatly dilutes the originally highly concentrated chrome liquor which had been added to the drum in small volume, thus making possible the diffusion of the now diluted chrome liquor into the skin fiber.

A convenient one-bath method is that in which both pickling and tanning are performed in the same drum, as described above. This preference is because such a process may lend itself to better control than do other methods, and permits the production of high-quality leathers as well. Consequently, most of this chapter will deal with the various factors involved in such tannage and, finally, with their theoretical interpretation. But before proceeding, it will be well to describe the general steps involved and the main factors which result.

The bated skin is pickled in the revolving drum until equilibrium is reached. The point of equilibrium is usually determined as a function of the pH value of the exhausted pickle solution, as indicated by the glass electrode. Any necessary adjustment to a predetermined standard pH value may be made at this point by the addition of the required amount of acid or alkali. The pickled skins are now ready for tanning. The required amount of chrome liquor of the proper basicity is slowly added through the gudgeon of the revolving drum. The rate at which the chrome liquor is added varies with the preference and experience of individual tanners, and with the general process employed.

The drumming is continued until tannage is judged to be complete. In some processes tannage is not completed until, near the end of the process, a small amount of a mild alkali (such as sodium or ammonium bicarbonate or borax) has been added to the drum. When we speak of "completeness" of tannage we mean that sufficient chrome has been fixed by the skin and that the basicity of the resulting leather is of the proper and predetermined value. The alkali addition noted may be necessary to attain the required chrome fixation and leather basicity.

It is now known that these two factors are a direct function of the over-all basicity of the drum contents. By "over-all" basicity is meant the basicity of the entire drum contents, including all pickle acid. As the over-all basicity is raised (*e.g.*, by the addition of alkali) the ability of the skin to fix chrome is proportionately increased, and so also is the basicity of the tanned skin. In some tannages the addition of alkali is found not to be necessary, because the over-all basicity of the drum contents is already sufficiently high. As will be discussed later, the skin attempts to come into equilibrium with its surroundings, and if the tanning period is sufficiently lengthened the tanned skin attains the same basicity as the over-all basicity of the drum contents. This does not usually happen in practical tanning because the tan drum is not run long enough to attain complete equilibrium. The tanner usually considers chrome leather to be tanned when it does not curl or lose in area upon subjection to hot water. But this does not necessarily mean boiling water. Many of the best American chrome upper leathers are considered properly

tanned when they stand a set standard of temperature, which may be appreciably below 212° F. Certain other good leathers will actually stand boiling in water. The very important subject of the resistance of chrome leather to heat will be discussed later in this chapter.

If we now attempt to summarize the important factors in drum tanning, the following are found: nature of pickling treatment and composition of the pickle solution; effect of all the various ions present in the tan drum; relation between pounds of hide substance and pounds of liquor in the drum; temperature of the drum contents during tanning; the percentage of Cr_2O_3 given per unit of hide substance; over-all basicity of total drum components and their pH value; composition of the chrome compounds present in the drum, together with the length of drumming period and the speed of the drum. All these factors influence the fixation of chrome and the characteristics of the final leather product. The successful tanner is one who strives to keep uniform the process he has found to be best suited for the leather he wishes to produce. However, such uniformity may be achieved only by strict chemical control.

Preparation of One-Bath Chrome Liquors

As has been noted, most chrome leather is produced by the one-bath method. By this it is meant that the pickled skin or hide is tanned directly by means of a basic chrome solution. The manner in which such basic solutions are prepared greatly influences the character of the leather produced. There are numerous methods of reduction, but all involve the reduction of chromium from the hexavalent to the trivalent state. The two most important commercial methods employ either glucose or sulfur dioxide as the reducing agent, and these two methods will now be discussed at some length.

Glucose Reduction. In 1897, H. R. Procter[62] suggested the addition of sucrose or glucose to an acidified bichromate solution, whereby reduction is effected and basic chrome compounds are obtained. Procter pointed out that the organic by-products of the reaction would no doubt influence the tanning qualities of the resulting chrome liquor. A large proportion of the basic chrome liquors used today are glucose-reduced. The method of preparation usually consists of dissolving sodium bichromate in a lead-lined tank containing a stirring device; the proper amount of sulfuric acid is then added and this is followed by the very slow addition of the requisite amount of glucose. The glucose must be added very slowly to prevent the reaction from proceeding at a too violent rate. The completed "liquor" may show a specific gravity of 48° Bé. and is usually allowed to cool and to age for several days; after this it may be diluted to 40° Bé. (or whatever gravity is desired) and should then be allowed to age for at least one week before use. The reason for the ageing is that time is required for the various chrome compounds formed to become stable in composition.

The theoretical reaction of the reduction described above, employing sucrose as the reducing agent, would be:

$$8Na_2Cr_2O_7 + 24H_2SO_4 + C_{12}H_{22}O_{11} \longrightarrow 8Na_2SO_4 + 16Cr(OH)SO_4 + 27H_2O + 12CO_2$$

This reaction, requiring 16.3 per cent of sucrose on the anhydrous bichromate weight, yields a 33⅓ per cent basic chrome sulfate, assuming the sucrose has been completely oxidized to CO_2 and H_2O. Such complete oxidation does not occur under the manufacturing conditions of the tannery, where factors of temperature, concentration of unreduced chromic acid, etc., interfere. As a result, other oxidation products of sucrose or glucose are formed which may greatly influence the composition and tanning behavior of the chrome compounds produced. The amount and kind of such products, and their ultimate effect upon the nature of the chrome liquor, vary with local tannery conditions of manipulation. The products also vary with the sequence of mixing the original reactants, as will be shown. When organic acids are formed, they decrease the basicity of the liquor below the calculated value, based upon the inorganic acid used. They may also penetrate the chrome complex and radically change its tanning behavior and render it less astringent. Such compounds are often termed "masked," because they possess increased resistance to the precipitating effect of added alkali. It is because of the phenomena just described that glucose-reduced liquors possess tanning properties different from those obtained if the reduction is brought about by the much simpler process employing sulfur dioxide.

General tanning experience indicates that plumper upper leather is produced by glucose-reduced liquors than by liquor reduced with sulfur dioxide. Many other organic reducing agents have been suggested from time to time, such as glycerin, spent tan bark, wood shavings, leather shavings, sulfite cellulose liquors, etc. But since the reaction is very complicated at best and since glucose is comparatively cheap, its use has not been displaced by the less well understood organic materials noted. Their employment may be considered, however, if conditions, such as those of war, make it difficult to secure glucose.

There are on the market a number of prepared basic chrome compounds, in dry form, which some tanners use in preference to preparing their own chrome liquors. These compounds cover a wide variety of composition and some of them are said to be glucose-reduced. The heating necessary for preparing solid extracts and for their subsequent solution is presumed to bring about changes in the composition of the chrome complex, and may affect its electrophoretic migration and its tanning behavior.

The first adequate quantitative study of the chemistry of glucose reduction was published by Stiasny and Ziegler[77] in 1931. They prepared glucose-reduced basic chrome sulfate liquors in which a number of factors of reduction

Table 169

Experiment	First Basicity Value	Second Value	Third Value	Total Organic Acid	Non-volatile Organic Acid	Volatile Organic Acid
A	33 3	33 4	27.0	6.3	0.0	6.3
B	33.3	33.1	25.1	8.2	0.0	8.2
C	43.3	43.4	33 0	10.3	0 0	10.3
D	33.3	30.7	18 6	14.7	2.7	12.0
E	33 3	31.3	16.5	16.8	1.9	14.9
F	43.3	39.5	26.9	16.4	3.1	13.3
G	33.3	31.9	21.6	11 7	1.0	10.7
H	33.3	31.3	23.0	10.3	1.4	8.9

were varied; the results are shown in Table 169. Liquors A, B and C were prepared by adding glucose solutions to a potassium bichromate-sulfuric acid mixture; liquors D, E, F and G by adding sulfuric acid to a bichromate-glucose mixture; liquor H was prepared by adding cold saturated bichromate solution to a sulfuric acid-glucose mixture. The proportion of bichromate to acid was such (with the exception of C and F) that the theoretical basicity of the finished liquors would be $33\frac{1}{3}$ per cent; C and F were 43.3 per cent. Liquors A, C, D, F and H were reduced with the minimum amount of glucose required for complete reduction, B and E were given 10 per cent excess glucose, and liquor G was reduced with a mixture of glucose and dextrin. The chrome content of all the liquors was 80 grams per liter. Reductions were carried out at the boiling temperature of the solutions for 90 minutes. The pH values of the finished liquors varied with the amount of acid employed and ranged between 2.85 and 3.40. Electric migration was determined after the liquors had aged for three weeks and was found to be cathodic in all cases.

It will be noted that three final basicity values are given in the table, which are marked 1, 2, and 3. The first is the calculated or theoretical basicity, based on the inorganic acid employed. The second value was obtained by determining the total acid present by means of the usual hot titration with NaOH, employing phenolphthalein as indicator. But before such titration was made the analytical chrome solution was boiled to drive off any free volatile acids which had been formed. The third basicity value was secured by means of the formaldehyde-barium chloride method, whereby all acid groups—both organic and inorganic—are presumed to be determined. The amount of both volatile and non-volatile organic acid formed during the various reductions is expressed in the table as a function of degree of change in basicity. Thus, the difference in the first and second basicity values measures the non-volatile organic acid formed, which in the examples described was found to consist exclusively of oxalic acid. The difference between the first and third basicity values indicates the extent of formation of both volatile and non-volatile acids.

Theis and his collaborators[86] extensively studied sugar reduction in a series of papers published in 1934-39. These investigations will now be

summarized and the reader is referred to the original articles for more detailed information.

The methods of reduction were varied along the lines pursued by Stiasny and Ziegler, as follows:

(1) Addition of sucrose to a solution containing potassium bichromate and sulfuric acid.

(2) Addition of sulfuric acid to a solution containing sucrose and bichromate.

(3) Addition of bichromate to a hot solution of sucrose and sulfuric acid.

In all cases the third reactant was added slowly (over a period of 15 minutes) to a boiling solution of the other two. The mixture was then heated for two hours. All liquors had a theoretical basicity of $33\frac{1}{3}$ per cent. Employing the general experimental scheme just described, the amount of sucrose used was varied from the theoretical requirement without excess to 50, 100, 150 and 200 per cent excess. At the conclusion of reduction, determination was made of the following oxidation products: carbon dioxide evolved, acetic, formic and oxalic acids, aldehydes, humic acids (insoluble organic matters), insoluble polymers of hydrocarbons, glyoxylic acid, and unchanged sucrose.

When sucrose was added to the acid-bichromate mixture, scheme (1), the amount of CO_2 evolved equals some 70-75 per cent of the theoretical amounts of the equation: $8K_2Cr_2O_7 + 24H_2SO_4 + C_{12}H_{22}O_{11} \rightarrow 12CO_2 + 16CrOHSO_4 + 8K_2SO_4 + 24H_2O$. These authors point out that when the sucrose is added that it is the first portion only which encounters conditions that are favorable for the formation CO_2, and that there is no additional CO_2 evolution when the amount of added sucrose is increased. When acid is added to the sucrose-bichromate mixture, scheme (2), CO_2 evolution falls from 64 to 31 per cent of the theoretical as increasing amounts of sucrose are employed and under scheme (3) it ranges from 74 to 49 per cent of theoretical.

The general results of all these experiments may be best understood from Figure 141, from which it will be noted that carbon dioxide is the highest proportionate oxidation product and that its amount, as well as the amount of the various organic acids and formaldehyde formed, is a function of the manner in which the various reactants are added. The results illustrated in Figure 141, were obtained by collecting and determining all the volatile products normally evaporated off and by determining the remaining volatile and non-volatile organic compounds present in the finished chrome liquors. When reduction is performed in an open system, as in tannery practice, much of the volatile products formed escape into the atmosphere, as would be expected.

Theis and his collaborators found that, in general, the theoretical oxidation equation was most nearly approximated when sucrose was added to a boiling bichromate-acid solution, and at the highest bichromate concentration. They

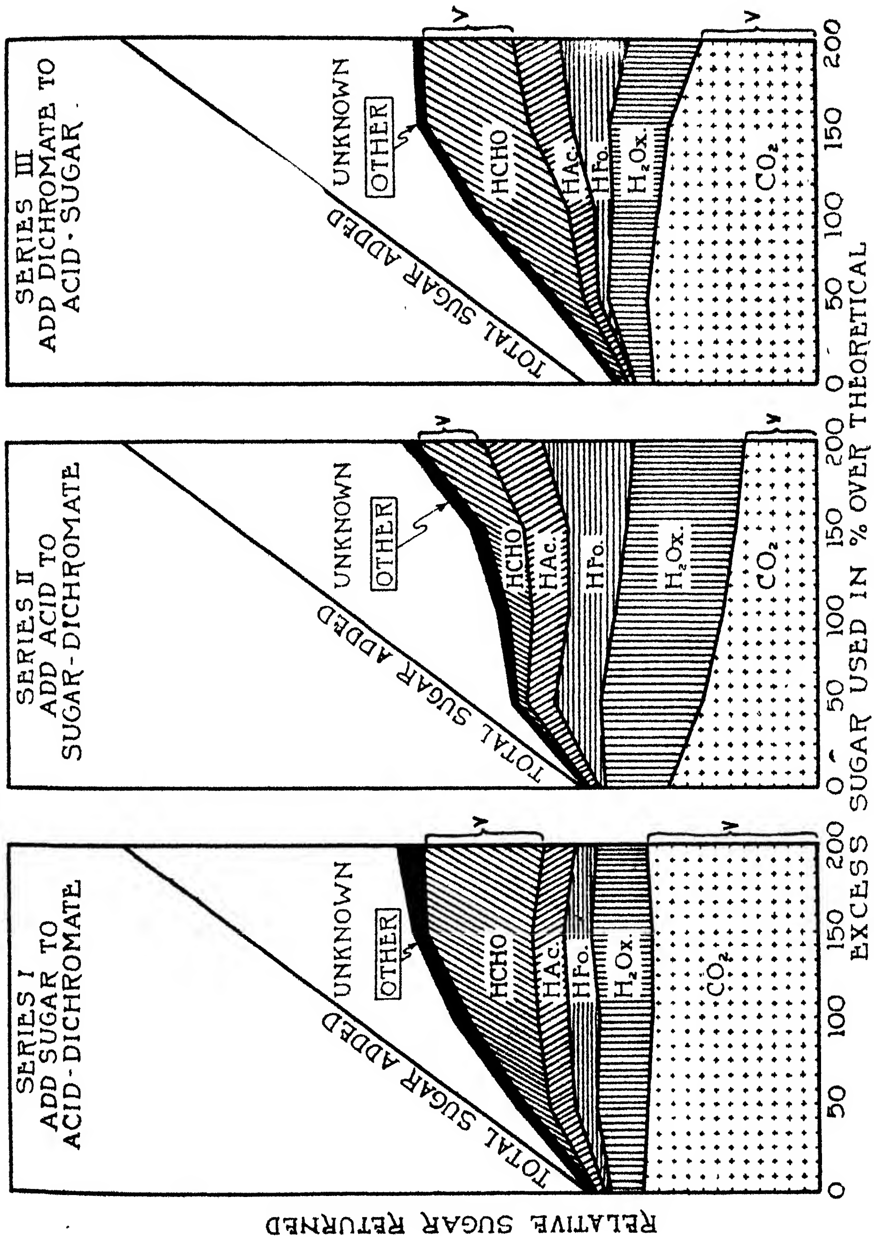

SERIES I
ADD SUGAR TO
ACID-DICHROMATE
SERIES II
ADD ACID TO
SUGAR-DICHROMATE
SERIES III
ADD DICHROMATE TO
ACID-SUGAR
TOTAL SUGAR ADDED
UNKNOWN
OTHER
HCHO
HAc.
HFo.
$H_2Ox.$
CO_2
0 50 100 150 200
EXCESS SUGAR USED IN % OVER THEORETICAL
RELATIVE SUGAR RETURNED

Figure 141

further found that masking agents are best produced when oxidation conditions are least drastic.

Sulfur Dioxide Reduction. In 1907, Appelius and Schall[2] described the reduction of bichromate with sulfur dioxide. The process was later suggested by both Balderston[5] and Procter.[63] This method is very simple to manipulate, consisting of the introduction of sulfur dioxide into an aqueous bichromate solution and heating the reduced chrome liquor to remove any excess, residual sulfur dioxide. The reaction may be dogmatically expressed as follows: $Na_2Cr_2O_7 + 3SO_2 + H_2O \rightarrow 2Cr(OH)SO_4 + Na_2SO_4$. In this way a $33\frac{1}{3}$ per cent basic chromium sulfate is obtained, without any of the organic by-products of the glucose reduction method. Stiasny and Gergely[78] have shown, however, that the equation given above does not necessarily express the course of the reaction, even though the end products may be as noted. According to these authors, the course of the reaction varies with the concentration of the solutions employed and with the temperature of reduction, as will be seen.

When a dilute solution of potassium bichromate, 10 grams per liter, is reduced in the cold with aqueous sulfur dioxide, the latter is completely oxidized to dithionate, thus: $K_2Cr_2O_7 + 7H_2SO_3 \rightarrow K_2SO_3 + Cr_2(S_2O_6)_3 + 7H_2O$. The chromium is now entirely in a cationic form. The liquor gives a precipitate with ammonia, but not with barium chloride, indicating that the anion of the complex chrome salt is not SO_4 but S_2O_6. If such a solution is boiled, the dithionate is decomposed, thus: $Cr_2(S_2O_6)_3 \rightarrow Cr_2(SO_4)_3 + 3SO_2$. The normal sulfate is now decomposed by the sulfite (which was simultaneously formed with the dithionate) yielding a $33\frac{1}{3}$ per cent basic chrome sulfate: $Cr_2(SO_4)_3 + K_2SO_3 + H_2O \rightarrow 2Cr(OH)SO_4 + K_2SO_4 + SO_2$.

When a higher concentration of bichromate is employed, say 50 grams per liter, and aqueous sulfurous acid reduction is performed at a low temperature, the resulting chrome liquor contains both anodic and cathodic chrome complexes. Precipitation occurs immediately upon addition of $BaCl_2$, but only after standing or heating upon addition of HCl and $BaCl_2$. These authors conclude, therefore, that the freshly prepared liquor does not contain free SO_4 ions. These appear only as they emerge from the anionic complexes, with the formation of cationic complexes. If the bichromate concentration is further increased (130 grams per liter) and reduction is performed at low temperature with gaseous sulfur dioxide, the resulting liquor contains a greater proportion of anodic complexes. It remains masked against the action of HCl and $BaCl_2$ for a longer time than liquors made from the lower bichromate concentrations. When a saturated bichromate solution (containing excess solid bichromate) is reduced at low temperature, with gaseous sulfur dioxide, a chrome compound is formed which migrates anodically almost exclusively. It remains masked against the precipitating action of $HCl/BaCl_2$ even after six months of aging. Its formation may be represented thus: $Na_2Cr_2O_7 +$

$3SO_2 + H_2O \rightarrow [Cr_2(OH)_2(SO_4)_3]Na_2$. When diluted it decomposes, first yielding a neutral, one-third basic complex ion, $[CrOHSO_4]$, and later a one-third basic complex cation, $[CrOH]^{++}SO_4^{--}$.

It may be assumed that under the manufacturing processes of the tannery, the end products of the sulfur dioxide reduction processes are, essentially, one-third basic chrome sulfate and sodium or potassium sulfate. But the experimental studies described above illustrate how complicated the course of the reaction may be, and they emphasize the necessity of uniformity in methods of reduction. A great deal of practical scale experimentation has been performed whereby various masking substances have been added to sulfur dioxide-reduced liquors, in an effort to simulate the leather produced with glucose-reduced liquors. While this information is not readily available for publication, it may be stated that many successful results have been reported.

Among other inorganic reducing agents used in the preparation of one-bath liquors there may be mentioned sodium bisulfite and sodium thiosulfate. The chemistry of such reduction processes has been investigated by Stiasny[76] and his collaborators.

Aging of Chrome Liquors

It is generally believed that all one-bath chrome liquors, especially those reduced with organic agents, should be allowed to age after they are made and before they are used. Most tanners allow their chrome liquors to age for at least three days and, preferably, for a week or more before using. But there is no general agreement as to the exact aging time required nor how such would be determined. Nor has any entirely convincing evidence been advanced to show that leather characteristics or quality may be correlated with time of liquor aging. We ourselves have been unable to find any difference in the extent of chrome fixation of leather, or in leather basicity, when a glucose-reduced liquor is used immediately or is allowed to age up to eight days before tanning under carefully controlled experimental conditions. Chrome content and leather basicity do not necessarily tell us anything as to leather quality. In discussing the point with many successful chrome tanners, one receives conflicting statements; one tanner may state that there is no difference in leather quality due to time of chrome-liquor aging, whereas another tanner finds considerable difference, particularly in leather fullness and uniformity. There can be but little doubt, however, that aging all one-bath chrome liquors for a week is a sound procedure. The theoretical reason underlying it is found in the fact that chrome liquor is a heterogeneous system which requires time in which to reach equilibrium.

Some of the factors which influence or determine the equilibrium referred

to above have been studied by numerous workers; they include changes in pH value, olation degree, and the relative proportion of chrome-bound acid radical which is complexly or ionically held. These experimental studies will now be discussed.

The investigations of Stiasny,[77] Stiasny and Grimm,[78] and Stiasny and Balanyi[79] may be summarized as follows. Aging changes in both normal and basic chrome sulfate solutions were determined by the fluctuations in pH value, the olation degree (derived by means of the indicator method to be described), ionized sulfate derived by a modification of the benzidine method, and complexly held sulfate by means of Gustavson's pyridine method. A 10-gm Cr per liter solution of normal chrome sulfate made up cold showed the changes upon aging at room temperature given in Table 170.

Table 170

Time	pH Value	$\frac{\text{Complex bound } SO_4}{\text{Total } SO_4 \text{ bound to Cr}} \times 100$
Immediately	2.68	0.0
After 3 days	2.65	8.1
After 4 weeks	2.38	18.1
After 7 weeks	2.30	27.0

The acidity increase noted in Table 170 is attributed to olation, which has influenced the hydrolysis equilibrium and liberated free H_2SO_4. When a similar solution was made and boiled with a reflux condenser for three hours, cooled, and the determinations above were repeated, the results shown in Table 171 were obtained.

Table 171

Time	pH Value	$\frac{\text{Complex bound } SO_4}{\text{Total } SO_4 \text{ bound to Cr}} \times 100$
Immediately	1.39	58.1
After 2 days	1.41	50.8
After 2 weeks	1.49	48.1
After 7 weeks	1.73	37.1

The decreased acidity induced by aging the boiled solution is explained as a balancing of effects: the de-olation which occurred on aging tended to decrease acidity; the stronger hydrolysis of the acid groups emerging from the complex tended to increase it.

These authors then studied basic chrome sulfate solutions, that is, solutions of normal chrome sulfate made basic by the addition of NaOH, and of a concentration of 10 gm Cr per liter. The results are shown in Table 172.

The authors point out the greater penetration of SO_4 groups into the complex of basic chrome sulfates than in the case of the normal salt, as shown by a comparison of Tables 170 and 172; that the higher the basicity the more rapidly the SO_4 groups enter, but that the maximum degree of penetration

Table 172

Time after making basic	pH Value	$\frac{\text{Complex bound } SO_4}{\text{Total } SO_4 \text{ bound to Cr}} \times 100$
	12 per cent Basic	
5 min.	4.32	5.7
19 hours	...	...
34 hours	...	...
2 days	2.84	24.8
2 weeks	2.51	31.3
7 weeks	...	40.2
	33 per cent Basic	
5 min.	...	...
19 hours	3.63	29.1
34 hours	...	. .
2 days	3.10	36.4
2 weeks	2.99	37.6
7 weeks	2 93	39.9
	50 per cent Basic	
5 min.	...	...
19 hours	...	..
34 hours	3.51	39.4
2 days	3.34	38.9
2 weeks	3.32	39.9
7 weeks	3.31	38.7

Table 173. Degree of Olation of 33.3% Basic Chrome Sulfate at Room Temperature.

After	0 1% Cr	0 2% Cr	0 5% Cr	1 0% Cr	2 0% Cr	3 0% Cr
1 hour	19.0	19.0	17.1	16.6	15.0	13.2
5 hours	54.0	55.6	60.1	62.5	63.8	64.9
24 hours	75.1	76.9	77.8	79.9	82.5	84.3
5 days	82.9	84.5	87.5	89.3	91.6	94.0
4 weeks	85.6	88.2	91.0	93.2	95.3	97.1
			At 40°			
1 hour	77.9	80.3	83.1	84.3		
5 hours	88.1	90.5	92.4	94.2		
24 hours	92.0	94.3	96.3	97 5		
5 days	92.0	94.3	96.3	97.5		
			At 75°			
1 hour	92.9	95.6	97.3	98.5		
5 hours	100.6	99.2	101.3	100.6		
24 hours	100.6	101.3	101.3	100.6		
		50.0% basic.	Room Temp.			
1 hour	35.2	35.9	36.8	37.7		
5 hours	60.3	61.2	63.8	64.9		
24 hours	73.7	75.6	78.7	79.9		
5 days	85.2	87.1	89.7	91.1		
4 weeks	89.1	91.1	95.2	96.8		

appears to be independent of the basicity degree. And they stress the fundamental difference in the behavior of basic chrome chloride and basic chrome sulfate, in that the chloride ions are always ionically held by the former, whereas some of the sulfate ions are always complexly held by the latter. The chemistry of the basic chrome chlorides is consequently much simpler than that of the basic sulfates.

Stiasny and Königfeld[80] have studied the influence of basicity, time, temperature, and concentration on the degree of olation of both basic chrome chlorides and sulfates, employing the indicator method to be described. Their results for some basic chrome sulfates are shown in Table 173.

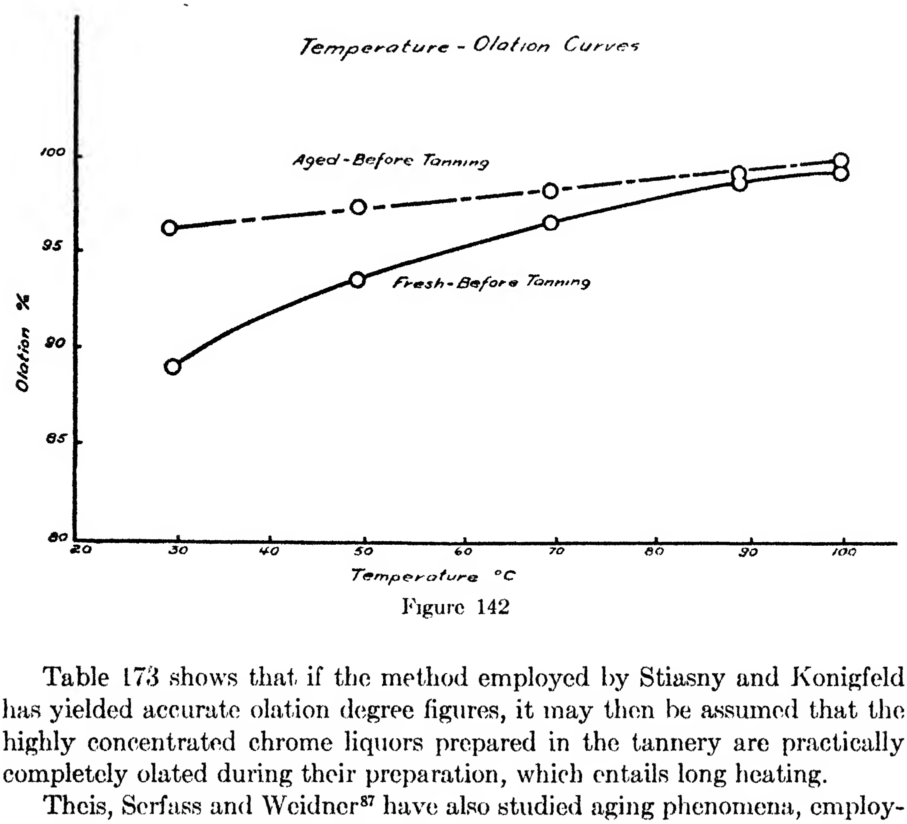


Figure 142

Table 173 shows that if the method employed by Stiasny and Konigfeld has yielded accurate olation degree figures, it may then be assumed that the highly concentrated chrome liquors prepared in the tannery are practically completely olated during their preparation, which entails long heating.

Theis, Serfass and Weidner[87] have also studied aging phenomena, employing a dry chrome extract which was prepared by evaporating a 35 per cent basic glucose reduced chrome liquor. Various solutions containing 1.0 per cent Cr_2O_3 were made by dissolving the dry extract at temperatures varying from 30 to 100°; these temperatures were maintained for one hour to insure complete solution at the lower temperatures.

The olation degree of solutions prepared as above, and containing 1.0 per

cent Cr, was determined immediately after the original solutions had cooled and again after they had aged five days at room temperature. The conductometric method was employed. The results are shown in Figure 142. It will be noted from the figure that both fresh and aged solutions were already highly olated, and that all solutions showed practically complete olation at 100°.

The percentage of free acid of the various solutions was determined by a conductometric titration of 50 ml of 0.1 per cent Cr dilution with NaOH. The results are illustrated in Figure 143, which shows a low initial free acid

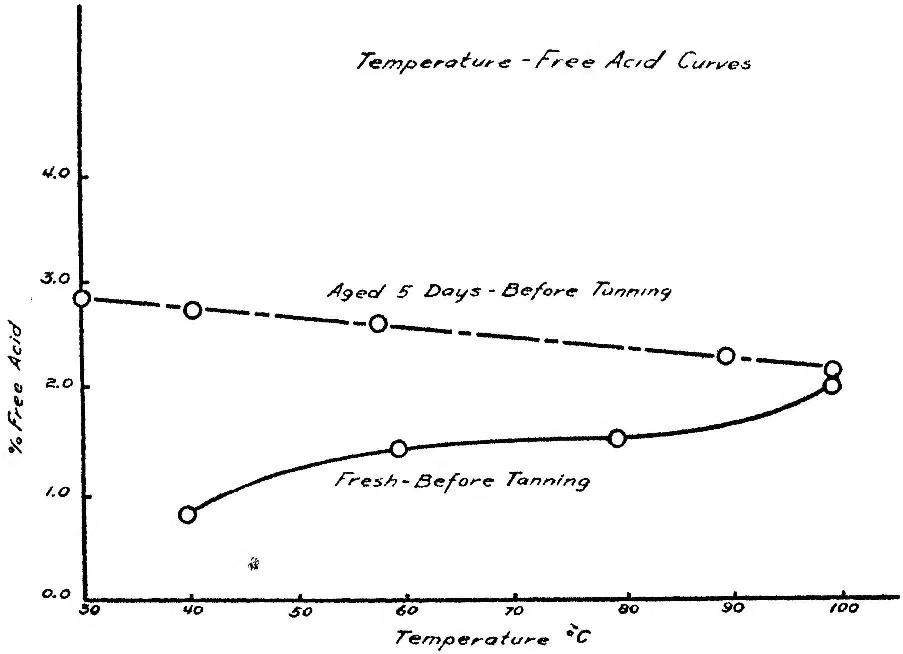


Figure 143

content in the freshly prepared solutions; this value increases with rising temperature. The aged solutions, however, show just the reverse; that is, a high free acid value at 30°, which decreases with rising temperature, until it equals the value of the fresh solution at 100°.

The effect of aging on the distribution of chrome-held sulfate radical was also determined by Theis, Serfass and Weidner. They dissolved the dry chrome extract described at 20°, diluted it to contain 0.75 per cent chromium, filtered it, and determined ionic sulfate as follows. To 25.0 ml of the chrome solution were added 35.0 ml of 0.2N $Ba(NO_3)_2$ and seven minutes later the solution was conductometrically back-titrated with 0.2N Li_2SO_4. Complexly

bound sulfate was thus obtained by difference. Portions of the freshly prepared 0.75 per cent chrome solution were allowed to age for 0, 2, 4, and 5 hours; on titration these showed 54, 49, 47, and 46 per cent respectively of the total chrome bound acid to be complexly held.

Küntzel, Riess and Königfeld[41] have discussed the phenomenon of the aging of basic chrome sulfate solutions. They divide aging into two phases: a primary hydrolysis whereby free acid and basic salt result and where equilibrium is quickly reached and, secondly, the aggregation of the basic salts thus formed to larger molecules, which are more acid-resistant. They point out that such acid-resistance may not be due merely to olation, as Stiasny has taught, but may involve the conversion of the olated compounds to the oxo state.

In considering the various phenomena of aging as discussed in this section, we would re-emphasize that it has not yet been clearly established that aging of chrome liquor and quality and characteristics of leather can be satisfactorily correlated. On the other hand there is no evidence that aging of chrome liquor is undesirable. When we attempt to evaluate the various constants which have been detailed in this section, it must not be forgotten that prepared tannery chrome liquors are highly concentrated systems and that all the experimental results described were obtained with greatly diluted solutions. We cannot be sure, for example, that the relation of ionic and complexly held acid sulfate found in a 0.1 per cent chromium solution is necessarily that of the highly concentrated tannery liquor from which it is made, since the equilibrium conditions of the two solutions are quite different.

Basicity of Chrome Compounds

Since basic compounds are the important chromium salts in tanning, a means of calculating and expressing their basicity value becomes necessary. Several methods of expressing basicity have been suggested, but the convenient one due to Schorlemmer has been generally adopted. According to this system, basicity is the percentage of the total Cr_2O_3 present which is combined with hydroxyl. Thus $Cr_2(SO_4)_3$ would have a basicity of zero and an acidity of 100; $Cr(OH)(SO_4)$ would be 33.3 per cent basic and 66.7 per cent acid; $Cr_2(OH)_4(SO_4)$ would be 66.7 per cent basic and 33.3 per cent acid, and $Cr(OH)_3$ is 100 per cent basic. The over-all basicity (that is, calculated on all chrome and all acid radical present) of a chrome liquor or of a chrome leather, may be conveniently calculated as follows:

$$\frac{\text{equivalents of acid } SO_4}{\text{equivalents of } Cr_2O_3} \times 100 = \text{Acidity}$$

Since both acid sulfate and chromic oxide are usually expressed in percentage, we can substitute these figures for equivalents in the above formula

and calculate the acidity by multiplying the per cent acid sulfate by 52.8 and dividing the result by the per cent chromic oxide. The acidity value thus obtained, deducted from 100, equals the basicity. In the case of the basic chrome chlorides the factor is 71.4.

It must not be overlooked that the usually expressed basicity value of a chrome liquor is computed from the total value of its acid components—that is, both the free acid and that combined with the chrome. And in the case of leather basicities, unless otherwise stated, the sum of both chrome bound and protein bound acid is employed.

Analytical and Experimental Methods. In order that the reader may better understand the experimental results to be described and discussed, it becomes necessary to outline briefly the methods whereby they have been obtained. This information will also facilitate the repetition or the extension of the experimental studies herein described when desired. Those who have followed the literature of chrome tanning will have noted that conflicting experimental results are often due to differences in experimental technique. We shall therefore describe the principal methods now employed in chrome tanning studies.

pH Value. The pH value of all aqueous solutions may be easily and accurately determined by means of a glass electrode. The ordinary type of glass electrode is unaffected by those ions which "poison" the hydrogen electrode, and it is accurate to a pH value of approximately 10.0.

Precipitation Point Value. This value was suggested by McCandlish in 1917.[49] It is useful not only in tannery control of chrome liquors but is of importance in many theoretical studies. As the term implies, it is a determination of the amount of alkali which must be added to an acid chrome liquor to bring about a permanent precipitation of the chrome it contains. Alkali is slowly added to the chrome solution (which is first filtered in the case of exhausted tan liquors) until a permanent turbidity appears. This method is satisfactory for routine tannery operations, where approximate accuracy may be sufficient. But when precipitation studies require highly accurate results, precipitation cannot be correctly judged with the naked eye, as Stiasny and Ziegler[81] have shown. These authors have employed a nephelometer, that is, a Zeiss step photometer with a cloudiness scale.

Chromium. There are several standard methods for the determination of chromium, but the authors have found that the simplest and quickest method is that suggested by Cameron and Adams.[11] This method is highly accurate and is as follows.

In the case of chrome liquors, 25 ml of the diluted liquor (containing 0.0015 to 0.0025 gram Cr_2O_3 per ml) is pipetted into a 125-ml Erlenmeyer flask. Add 10 ml perchloric acid (70-72 per cent), place a small funnel in the neck of the flask and heat moderately on the hot plate until the chrome is completely oxidized. Cool, transfer to a 500-ml Erlenmeyer flask and make volume up to approximately 150 ml with distilled water. Boil to remove chlorine, then add 5 ml of conc. hydrochloric acid and cool. Add 10 ml potassium iodide

(10 per cent) and titrate with $0.1N$ sodium thiosulfate until the yellow color has almost disappeared; add starch indicator and titrate to the end point. In the case of chrome leathers, weigh out 1.5 to 2.0 grams (depending upon Cr_2O_3 content) of sample into a 125-ml Erlenmeyer flask. Add 20 ml perchloric acid (70-72 per cent), cover with a funnel, and heat moderately on a hot plate When the chrome is oxidized, remove from hot plate and cool. Wash down any unoxidized chrome with distilled water and heat until white fumes appear. Cool and transfer to a 500-ml Erlenmeyer flask and dilute to about 150 ml. Boil to remove chlorine, add 5 ml of conc. hydrochloric acid and cool. Titrate for chrome as under chrome liquors.

It should be carefully noted that perchloric acid may not be safely used in the case of leathers containing appreciable amounts of easily oxidizable fatty or other organic material, since explosions may occur in such cases. This danger may be entirely obviated, however, by previously extracting the fatty matters, by ashing the leather, or by previous oxidation by means of nitric acid as described by Mertz.[60]

Acid Groups. Methods for determining acid groups vary with the material to be analyzed and the constants which are sought. Thus, in the case of a chrome liquor one may wish to determine any, or all, of the following possible acid radicals: total acid, free acid, acid complexly held (that is, inside the chrome complex), acid ionically bound to the chrome complex, and also the acid radical bound to an inorganic base, or in other words the neutral salt or salts present; in the case of chrome leather: total acid, acid complexly and ionically bound to the fixed chrome, protein-bound acid and, finally, that acid radical contained in any neutral salt present in the leather. The acid radicals in question may be only sulfate or chloride, or there may be others and particularly in the case of tannage with glucose-reduced liquors or where "masking" salts such as formate, acetate or oxalate, have been employed in the pickle or have been added to the chrome liquor.

Considering chrome liquors first, the total acid present is usually determined by boiling titration with $0.5N$ sodium hydroxide, using phenolphthalein as indicator; this is the official method of the American Leather Chemists Association. This method is fairly satisfactory and accurate but not entirely so; this is because a small amount of acid radical is usually occluded by the precipitated chrome and is not titrated. Several methods have been suggested for differentiating the distribution of the total acid radical and these methods will now be discussed. The subject has been especially investigated by Schindler and Klanfer,[72] by Stiasny and his co-workers,[76] by Ackermann[1] and by Atkin and Chollet.[3] All these studies relate more particularly to basic chrome sulfate liquors.

Sulfuric acid differentiation methods have been conveniently summarized by Atkin and Thompson,[4] as follows. There are four types of SO_4 groups to be determined: (a) groups complexly held by the chrome, (b) groups ionically held by the chrome, (c) ionic groups in the free sulfuric acid formed by hydrolysis and, (d) ionic groups resulting from sodium sulfate or other netural sulfates present. The sum of (a) + (b) + (c) is obtained by hot titration.

The neutral SO_4, (d), is determined by filtering the solution obtained in the hot titration and washing the precipitated chrome. The filtrate and washings are combined, are evaporated to proper volume, and the total SO_4 radical present is determined by means of benzidine (hydrochloric acid method) whereby all SO_4 radical, that is, (a) + (b) + (c) + (d), is obtained. The difference between this value and the sum of (a) + (b) + (c) equals the neutral salt SO_4, or (d). (We have found that in the case of liquors containing no neutral salt other than sodium sulfate, more accurate results are obtained by determining sodium and calculating neutral SO_4 therefrom.) Value (c) is obtained by electrometric determination of the pH value of a 0.1 per cent chromium solution of the liquor, freshly prepared with *cold* distilled water. It is assumed that if the pH value found is 3.3, or greater, there is no free sulfuric acid present and that (c) is therefore zero. But if the value is lower than 3.3 the solution is electrometrically titrated with 0.1N NaOH to 3.3. The number of ml alkali required is a measure of (c).

Atkin and Thompson state the pH value in the absence of free sulfuric acid to be 3.3, as noted above. Stiasny, on the other hand, gives a value of 2.8. (And our experience has shown that the pH value of a zero basic chrome sulfate, at a dilution of 0.1 per cent, chromium is approximately 2.8 at equilibrium.) The value of ionic sulfate, (b), is determined as follows: a benzidine sulfate determination is made on the freshly dissolved cold solution of the chrome liquor, and this is a measure of (b) + (c) + (d). We already know the value of (c) and (d) and are thus in position to calculate (b). Value (a), the non-ionic sulfate in the chrome complex, may be obtained by difference. Atkin and Thompson stress that the scheme detailed above is for sulfates only and does not apply to liquors containing organic-acid radicals. We shall now discuss methods for determining organic radicals in chrome liquors.

In 1937 Theis and Weidner[88] studied the organic by-products of sugar reduction of bichromate and suggested the following methods for their estimation. (1) Carbon dioxide is determined by passing the gases evolved during reduction into caustic soda solution. Barium chloride is then added to a portion of the solution; the excess NaOH is then titrated using phenolphthalein as indicator; methyl orange is added and the barium carbonate titrated. This method does not, of course, include carbon dioxide remaining in the chrome liquor. (2) Oxalic acid is determined as follows: excess of sodium acetate is added to the chrome liquor on the assumption that the acetate radicals will replace complexly held oxalate radicals. The solution is slightly acidified with acetic acid and excess calcium chloride is added. Calcium sulfate precipitates first, followed by calcium oxalate. The solution is properly filtered, the precipitate dissolved in 2N H_2SO_4 and titrated hot with $KMnO_4$. The oxalic value obtained may include both mono- and other dicarboxylic acids. (3) Formic acid is determined by steam distillation of the

acidified chrome liquor into boiling $BaCO_3$, estimating the formic acid by Finck's method; that is, by reducing mercuric chloride to the mercurous state. (4) Acetic acid and other volatile acids (in addition to formic) are determined by steam distillation of part of the distillate from (3) into $0.1N$ NaOH after having first oxidized the formic acid by warming with mercurous oxide.

Burton and Taylor recently suggested the following method for determining all organic radicals present in glucose-reduced liquors. Total acid groups are determined by hot titration with NaOH, and the total sulfate by benzidine titration. Organic radicals are determined by exactly neutralizing with NaOH, then evaporating and heating to convert organic radicals to carbonate, which is then titrated. This method does not differentiate the type of organic radical, nor is it applicable to liquors to which masking salts have been added. In 1920, Thomas[99] suggested the determination of acid sulfate in chrome sulfate liquors by conductometric titration with barium hydroxide. This method yielded results which were practically identical with those obtained by gravimetric means. They indicated that results obtained by hot titration were too low, due to absorption of sulfate radical by the precipitated chrome. Shuttleworth[74] has suggested a conductometric method of differentiating and estimating the various radicals present in different types of chrome liquors. This method has been investigated by Burton and Taylor,[10] who found that the value of neutral salts thus determined is too low, as is also the basicity of the liquor. It must be borne in mind that the great dilution of chrome liquor necessary for accurate conductometric measurements may bring about changes in the position of its acid radicals.

Accurate knowledge of the distribution of acid radicals in chrome leather is of importance. But our knowledge of this subject is in not very satisfactory shape, as we shall see. This is because adequate analytical methods are lacking. Early investigators recognized that there are three possible types of acid combination in chrome leather: acid combined with the hide substance, termed "protein bound," acid complexly bound to the fixed chrome and acid ionically bound to the fixed chrome.

In 1920 Thomas suggested a method for determining total sulfate groups in leather and this method has long been the official method of the American Leather Chemists Association; it is as follows: One gram of ground leather is heated in a bath of boiling water for two hours with 200 ml $0.1M$ potassium or sodium di-hydrogen phosphate. The flask is then removed and cooled to room temperature and the contents are made up to volume with distilled water. An aliquot portion is then treated with hydrochloric acid and barium chloride. After standing for three hours it is filtered and the precipitate is well washed with hot water; it is then ignited and weighed and the per cent sulfate calculated. In order to determine neutral sulfate (derived from sodium sulfate present) the procedure noted above is repeated with a duplicate

leather sample, except that water is used instead of the phosphate solution, an aliquot portion being titrated with $0.1N$ sodium hydroxide with methyl orange or para-nitrophenol; and this value is deducted from the total sulfate precipitated in the water extract. In this way any hydrolyzed acid sulfate is not included as neutral. The difference between the two values thus obtained is the total acid sulfate. This useful method tells us nothing about the manner in which the acid sulfate is distributed in the leather. When great accuracy is necessary, the sodium content of the leather should be determined and the neutral sulfate calculated therefrom, the sodium present in the skin substance used in making the leather in question having been determined and deducted.

Riess and Papayannis[68] have suggested the following method for determining all the acid groups present in chrome leather, including all acid radicals present in addition to sulfate or chloride. The ground leather (2.5 grams dry matter) is heated for one hour at 60° on a water bath in a flask containing 50 ml $0.1N$ ammonia and fitted with a reflux condenser, closed with a soda lime tube. The solution is then filtered into a measured quantity of $0.1N$ HCl; the leather is washed with water at 60° and finally with boiling water and filtrate and washings are mixed. A control solution is prepared containing the same amount of N HCl and of the same volume as the combined filtrate and washings, but with the leather omitted. Five ml. of 40 per cent formaldehyde are now added to each of the two solutions. They are then titrated with 0.1 or $0.2N$ NaOH. The difference in the titration value of the two solutions is a measure of the total acid groups in the leather. It will be understood that any ammonium salts present in the leather will be determined as acid. Different acid radicals may be identified and determined in the combined filtrate and washings described above. If the leather contains oxalate groups there will be a dechroming action due to ammonium oxalate. In such case part of the combined ammoniacal extract and wash waters may be oxidized with Na_2O_2, acidified with acetic acid and treated with $CaCl_2$ by boiling. The precipitated calcium oxalate is filtered off and is then dissolved in H_2SO_4 and titrated with $KMnO_4$.

In 1926[18] Gustavson posed the following question: "Does the combined acid, obtained upon analysis of the leather, exist in direct combination with the skin protein or is a part of the total amount of the analytically obtained acidity present in a chrome complex?" He then went on to suggest that if the bound acid was divided between protein and chrome, it was to be expected that the hydrolysis rate of the two forms would be quite different. The experiments he then performed proved this to be true. Pieces of delimed calf skin were pickled with H_2SO_4 and Na_2SO_4 to contain respectively 3.66 and 3.14 per cent H_2SO_4 combined with the collagen. Corresponding pieces of this pickled stock were then tanned with basic chrome sulfate. After

tanning and washing to remove unfixed chrome and unfixed acid, they contained 7.52 and 5.88 per cent fixed Cr_2O_3 and 6.19 and 5.53 per cent total fixed H_2SO_4, respectively, all based on collagen. The basicities of the two leathers were thus 57.50 and 51.30 per cent. Portions of the two pickled specimens and of their corresponding leathers were then placed in bottles containing distilled water, each bottle containing the equivalent of two grams of material on collagen basis and 300 ml water. The four bottles were rotated for 48 hours and the hydrolyzed and diffused acid in the external solution was neutralized at frequent intervals with 0.1N NaOH, using methyl orange as indicator. At the end of 48 hours' agitation both pickled specimens had given up all their combined acid. The two leather specimens—which had received the same 48-hour neutralizing treatment as the pickled specimens—contained, however, 4.92 and 3.73 per cent H_2SO_4. Gustavson reasoned that, since all the protein-bound acid of the pickled specimens had been removed during the 48-hour treatment, that which remained in the leather could only be chrome-bound. And it is important to note that the basicities of the leathers containing chrome-bound acid only were 66.2 and 67.2 per cent, respectively.

The method just described is termed the "neutralization method." Merrill, Niedercorn and Quarck[56] have repeated and extended Gustavson's experiments; they found that chrome-bound acid is appreciably hydrolyzed during the early hours of neutralization and that therefore no exact differentiation of the two types of bound acid is possible. But they concluded that, with slight modification of Gustavson's experimental procedures, the method was sufficiently accurate to be useful. And we would add that, with all its limitations, this method is the soundest that has been suggested for differentiating protein-bound and total chrome-bound acid in chrome leather. It does not differentiate complex and ionic chrome-bound acid.

Since the neutralization method described above is time-consuming, Gustavson[19] later suggested shaking the leather for a given period in a solution of pyridine, whereby all protein-bound acid is assumed to be removed. The leather thus treated is then washed with distilled water to remove neutral sulfate, and the sulfate remaining in the leather is determined and assumed to be chrome-bound. But Merrill, Niedercorn and Quark[56] found that this method yielded values for chromium-bound sulfate that were much too low. And they showed that the amount of chrome-bound acid determined by the method depended upon the total acid sulfate content of the leather, regardless of the state of its combination.

In 1934 Küntzel, Riess, Papayannis and Vogel[42] suggested the following method for differentiating the various sulfate or chloride radicals present in leather tanned with basic chromium sulfate or chloride liquors. Total acid present in the leather (a) was determined by means of the formaldehyde-

ammonia method described above. Protein-bound acid (b) was determined by soaking such an amount of ground leather (which had been dried and aged for eight days) as would contain 2.0 grams hide substance in 25 ml of distilled water for 0.5 hour; 25 ml 0.2*N* HCl was then added and the mixture was shaken for one hour at room temperature. The mixture was then filtered and an aliquot portion was titrated to pH 5.5 for residual acid using a mixture of methyl red and methylene blue as indicator, the amount of acid absorbed by the leather being determined by difference. Acid absorption was determined for untanned hide powder using the same method, and was found to be 0.927 milliequivalent per gram hide substance. Deducting the amount of acid which the leather bound from this value of 0.927 (assumed to be the maximum acid-combining capacity of hide substance) was assumed to give the protein-bound acid value of the leather. It was further assumed that the eight-day aging given the leather prevented any reaction between the fixed chrome it contained and the acid with which the leather was treated. Acid ionically held by the fixed chrome (c) was determined by means of a modified pyridine extraction; hence it was assumed that ionically bound and protein-bound acid were simultaneously secured. Deducting the value obtained above for (b) yielded (c). The sum of (b) and (c) deducted from (a) yielded the complexly bound acid (d). Employing this method these authors found that leather tanned with either basic sulfate or basic chloride contained practically no ionically bound acid, substantially all the chrome-held acid being complexly bound. From these results they contend that the chrome complex in such chrome leathers is neutral in character. They mention having thoroughly washed with water the leathers in question before analysis, but evidently did not consider or determine what effect such rapid hydrolysis undoubtedly had upon the original acid distribution in the leather.

The above method has not proved to be very satisfactory in practice. Possibly this is due to the following obvious objections: the assumed maximum acid-combining power of the hide powder is much too low; combined acid was secured by the inaccurate "by difference" method which does not differentiate acid chemically fixed from that merely mechanically bound, and it is necessary to age the examined leather since otherwise acid rapidly combines with freshly fixed chrome. No insight is thus obtained as to the distribution of acid groups in the original, *unaged* leather.

In attempting to sum up the subject of determining the manner in which acid radicals are distributed in chrome leather it must be kept in mind we are dealing with an equilibrium between the various types described herein. We cannot be sure that the results we obtain, after disturbing the equilibrium, are representative of the original condition. For this reason it may be that no completely satisfactory method is possible.

Determination of Olation Degree. In 1932 Stiasny and Königfeld[80] suggested the following method for determining the olation degree of basic chrome sulfates and chlorides. It is assumed that the olated OH groups in these compounds are not easily titrated in the *cold* with acid, whereas all OH groups are made available for titration if the liquor is de-olated by boiling under reflux for one hour with acid. Back titration of the excess, unused acid in the two cases permits calculation of the percentage of the total OH groups present which were olated. These authors recommended employing 50 ml of a 0.1 per cent Cr solution in each case and 25 ml 0.1N HCl. A special indicator is recommended and titration is carried to an end point of pH 2.8, the pH value of a 0.1 per cent zero basic chromic chloride. In the case of the basic chrome sulfate, a greater quantity of 0.1N HCl must be added for de-olation; this is due to the strong tendency of the sulfates to olate when heated, and hence the need for a great excess of added acid. In view of this, Stiasny and Königfeld suggest that when dealing with sulfates the boiling be dispensed with and the total OH groups be calculated from the basicity of the liquor. (This latter method would not be generally applicable in the presence of organic chrome compounds or buffer substances.) Shortly before the publication of the method of Stiasny and Königfeld just described, Fasol and Überbacher[15] suggested the determination of olation degree by means of conductometric titration. In 1934, Theis and Serfass[89] made extensive studies of the phenomena of olation, employing conductometric methods, described by them as follows. The liquor to be analyzed is diluted to approximately 0.1 per cent chromium. To 50 ml of this dilution add 30 ml 0.1N HCl and the resulting solution is titrated immediately with 0.1N NaOH by the conductance method. The degree of olation of the sample may then be calculated from the usual formula. The sample is not titrated to pH 2.8, as in the method of Stiasny and Konigfeld, but to a "kink" point of the conductance curve. Serious discrepancies in results were obtained by these authors in attempting to apply the titration methods of Stiasny and Königfeld, even when the glass electrode was substituted for the indicator which had been recommended. Riess[69] has also found the method of Stiasny and Königfeld to yield erroneous results, but obtained essentially similar olation values by both potentiometric and conductometric titrations. However, from all the available evidence, including the studies of Fasol and Überbacher, Theis and Serfass, Riess, and Perkins and Thomas,[66] it would appear that the conductometric method yields the most accurate and reproducible results.

Hide Substance for Tanning Experiments. A large proportion of published experimental tanning studies has been made with hide powder. This material is easily obtained, is very uniform in composition and physical state and, for certain purposes, is entirely satisfactory. For many experimental purposes it is, however, unsatisfactory. This is because it has entirely lost

the original physical structure of the hide or skin from which it is ground. As a result, experimental studies with hide powder may bear little or no relation to the actual processes of the tannery; and it is just these processes which leather chemistry attempts to explain. On the other hand, experimental pieces cut from occasional hides or skins may not be uniform from one skin to another, nor, indeed, from different area locations of the same skin. In view of this, and in order to obtain material of constantly uniform chemical composition and physical state, and which will be typical of the skin in actual tanning processes, McLaughlin, Cameron and Adams[50] have suggested the following. Whole, bated calf skins (or goat skins) are brought to a pH value of 5.0 by the slow addition of small portions of hydrochloric acid and are then washed free of neutral salts, when they show a pH value of about 6.5. They are then cut into strips 0.5 inch wide and are placed in frequently changed dry acetone until thoroughly dehydrated. The acetone is allowed to evaporate and the now dry strips (containing 10-12 per cent moisture) may be cut into pieces of convenient size and the pieces from the entire lot are then well mixed. We have found pieces 0.5 inch $\times$ 0.5 inch to be admirably suited for experimental studies.

Removing Uncombined Matters from Leather. When hide substance is tanned in solutions of chromium compounds and is then removed from the tan liquor, it contains both combined and uncombined chrome and both combined and uncombined acid. If we desire to determine the fixed chrome only, and are not concerned with the basicity of the leather, we can remove all uncombined matters by simply washing the leather in cool running distilled water for 48 hours. The fixed chrome is unaffected by such washing—in fact, it becomes increasingly insoluble. This method of removing uncombined chrome is entirely satisfactory and reproducible for leather tanned in all of the usually employed chrome liquor concentrations. But it is not satisfactory in the case of leathers tanned in highly concentrated chrome liquors; washing such leathers brings about an artificial deposition of chrome in the leather, due to the rapid hydrolysis of the uncombined chrome liquor inside the leather when brought into contact with water. This subject will be further discussed at the end of this section.

If we wish to determine accurately the basicity of leather at completion of tannage, means other than washing must be employed. This is because the combined acid (both protein-bound and chrome-bound) will obviously be rapidly hydrolyzed and removed by contact with water. The problem, therefore, is to remove completely all uncombined chrome and all uncombined acid, leaving the combined chrome and combined acid, both protein- and chrome-bound, intact and undisturbed. Early workers were forced to adopt compromise methods for removing unfixed chrome and unfixed acid. They did this by giving the leather a short wash in water, hoping that such abbre-

viated washing would remove all uncombined matter without greatly changing the value of combined acid. But as Gustavson[18] and also McLaughlin and Adams[51] have shown, even a short water wash removes very appreciable amounts of combined acid and thus renders inaccurate any leather basicities so obtained. In view of this condition, Cameron, McLaughlin, and Adams[12] devised the pressing method as follows.

The tanned and drained skin squares are pressed twice in a hydraulic press at sufficient pressure to remove all uncombined matters. The ordinary laboratory type of hydraulic press is satisfactory. The squares are placed in a single layer upon, and between, 0.125-inch stainless steel plates of the proper size to fit the press platen, blotting paper having been introduced beneath and above the wet leather to absorb the expressed liquid. The press is then brought to the required pressure and held there for one minute. The plates are then removed; the pressed leather specimens are withdrawn, placed between fresh blotting paper between the steel plates, and are again pressed for one minute. The pressure required for removing all uncombined matters should be determined for the leather material under examination. It must be remembered that the actual pressure exerted varies with the number of square inches of material being pressed; thus the press gauge may read 5000 lbs, but if two square inches of leather are in the press the actual pressure exerted per square inch will be only 2500 lbs. There is a wide range of pressures which are effective for removing the uncombined chrome and acid in wet chrome leather. Thus McLaughlin and Adams[51] have found that leather which contained 16.50 per cent Cr_2O_3 after pressing at 10,000 lbs showed 16.54 at 5000; 16.57 at 2500; 16.60 at 1250; 16.85 at 625 and 16.80 per cent at 312 lbs. Many experiments have shown that a pressure of 5000 lbs is ample for the complete removal of uncombined chrome and acid. This statement refers to leather tanned in ordinary chrome liquor concentrations. When excessively concentrated liquors are employed, pressures of even 10,000 lbs are unable to remove completely all uncombined matter. In such special cases we are, as yet, unable to derive leather basicities of unquestioned accuracy. We can, however, obtain reasonably accurate figures for fixed chrome by employing a combination of pressing and washing as described and discussed on page 453.

Basicity Inaccuracies Induced by Washing Leather. We have just stated that even a short water washing of chrome-tanned hide substance removes combined acid and renders inaccurate any leather basicities so derived. Gustavson[18] demonstrated that this statement is true, in the case of leather, by the following experiment. He secured specimens of two different lots of calf skin leather taken from the tannery and which had been well washed prior to sampling; such preliminary washing had, of course, removed a large portion of their combined acid, but enough remained to

illustrate the point under discussion. The leather was then cut into 1.0-mm squares and samples equal to two grams collagen were weighed into 500-ml flasks and 300 ml of distilled water added. The flasks were then rotated for 48 hours at room temperature and the hydrolyzed combined sulfuric acid in the water was neutralized at frequent intervals. The results are shown in Table 174.

Table 174

Hours Washed	Basicity of Leather No. 1	Basicity of Leather No 2
0.0	57.50	51.30
0.5	60.20	55.60
1.0	61.20	57.90
2.0	61.90	59.30
3.0	62.40	60.50
6.0	63.10	61.80
9.0	63.80	62.50
24.0	64.80	63.60
28.0	65.50	64.90
32.0	65.90	66.20
48.0	66.20	67.20

McLaughlin and Adams[51] have determined the effect of washing chrome-tanned hide powder upon its basicity. They tanned powder for 48 hours at 90° F and with constant agitation and in a 31.0 per cent basic chrome sulfate liquor which contained no neutral salts or organic acid. At the end of tannage the excess liquor was drained from the tanned powder, which was then placed in a cloth bag and pressed twice at 5000 pounds to remove all unfixed chrome and acid. (The pressed tanned powder contained 8.80 per cent fixed chrome on hide substance basis and showed a basicity of 31.0 per cent.) Ten gram portions of the pressed (undried) tanned powder were then spread on Buchner funnels and were washed with distilled water; the rate of washing was such that 83.3 ml of water passed over the powder per minute. Two different washing temperatures were employed; one series received water at 25° and the other series was washed at 70°. The results are shown in Table 175.

Table 175

Minutes washed	Ml water used	Leather Basicity washed at— 25°	70°
0	0	31.0	31.0
15	1,250	39.5	40.6
30	2,500	40.4	43.0
60	5,000	43 6	48.1
120	10,000	46.2	55.2
240	20,000	49.8	60.8

It is obvious from the results shown in Tables 174 and 175 that any basicities determined in washed leathers do not represent their true basicities at completion of tannage.

The accuracy, as well as the limitations, of the pressing method are illustrated in Table 177.

Tanning to Equilibrium. Many arguments in chrome-tanning theory have resulted from the fact that tanning experiments have not always been carried to equilibrium. Thus if duplicate chrome solutions are employed in tanning, but if in one case the tanning is performed for 24 hours at room temperature and in another for 48 hours at, say 90° F, entirely different leather results may be expected. That is, the amount of chrome fixed and the basicity of the leather in the two cases will probably show great differences, which will merely reflect the fact that in one case equilibrium was not approached but may have been reached in the other. It is not possible to state any set rule of tanning time; this will vary as a function of temperature and with the nature and concentration of the tan solution. But we have repeatedly found that final equilibrium is reached, at least in the case of all basic chrome sulfates of more than zero basicity, when the basicity of the leather becomes the same as the overall basicity of the tanning solution employed, including, of course, any and all pickle acid which may be present. Only negligible quantities of additional chrome will be fixed if tanning is extended beyond this point. In most cases tanning equilibrium is attained with continuous agitation at 19 rpm (the approximate speed of tannery drums) for 48 hours and at 90° F.

It is, of course, understood that in the case of practical operations in the tannery, leather is usually not tanned to the equilibrium state described above.

Having considered the various experimental methods involved in the study of chrome tanning, we shall now describe the experimental investigations upon which our knowledge of chrome tanning is based.

The Effect of Temperature in Chrome Tanning

As would be expected, the temperature at which chrome tanning is performed is of importance in both practice and theory. In practical tanning the skins are tumbled in a drum with a volume of liquor which is usually too small to cover and float the skins; for this reason the skins are subjected to a pounding action, and the resulting friction causes the temperature of both skins and liquor to rise. Or, if a neutralizing agent such as sodium bicarbonate is added to the drum, the heat of its neutralization will heighten the drum temperature. As the drum temperature rises, the rate of tanning increases, the composition of the chrome compounds present may change, and the character of the leather produced may be considerably affected. For these reasons, the chrome tanner endeavors to maintain uniform tanning temperatures. Laboratory chrome-tanning experiments are equally sensitive to temperature.

In 1929, Merrill and Schroeder[57] tanned strips of pickled calf skin with a commercial basic chrome extract at varying temperatures. This was done by placing skin and liquor in large bottles which were immersed in thermostatically controlled water baths in which the temperature was maintained at 10°, 20°, 30°, 40° and 50°C, respectively. The jars were not constantly agitated but were stirred by hand at 15-minute intervals during the first 8 hours of tannage and three times a day thereafter. At the end of the various tanning periods studied, the strips were removed, washed for one hour in running tap water to remove uncombined matters, and then dried and analyzed. The effect of temperature upon chrome fixation may be judged by comparing the fixations at the end of 50 hours, as found by Merrill and Schroeder. The percentages of Cr_2O_3 fixed on hide substance basis were: 8.5 per cent at 10°, 9.5 at 20°, 12.0 at 30°, 13.5 at 40° and 15.0 at 50°. As has been noted, these authors did not continuously agitate their tanning systems. But McLaughlin and Adams, in an unpublished investigation, repeated—with constant agitation—the experiments of Merrill and Schroeder, employing unpickled skin squares. They find that while the percentages of chrome fixation are higher, as would be expected with constant agitation, the relative effects of temperature found by Merrill and Schroeder are correct.

Otin and Alexa have also studied the influence of temperature upon chrome fixation, employing hide powder, and have found such effects to be large. But their investigations are of less significance because chrome fixations were obtained "by difference"; that is, they were calculated from analysis of the exhaust liquors and not from analysis of the tanned powders.

The Effect of Liquor Concentration in Chrome Tanning

One of the phases of chrome tanning to be first investigated was the effect of chrome liquor concentration upon the amount of chrome fixed by hide substance. In 1919, Baldwin[6] tanned hide powder with liquors of increasing concentration of a 35.0 per cent basic commercial chrome sulfate. Fixations of chrome steadily increased until a liquor concentration of 25.0 grams Cr_2O_3 per liter was exceeded, when a sharp drop in fixation occurred. A similar drop in fixation was found by Thomas and Kelly[100] and also by Gustavson and Widen,[20] all of whom employed hide powder and basic chrome sulfate liquors. The point of liquor concentration at which decreased fixation was found varied as a function of the liquor basicity. These authors variously explained the drops in fixations noted as possibly due to increases in the hydrogen ion concentration of the chrome liquors, to chemical changes induced in the hide substance being tanned, and also to chemical change induced in the basic chrome complex.

In 1934, Küntzel, Kinzer and Stiasny[43] suggested that the drop in chrome fixations found was caused by the increasing concentration of sodium sulfate

present in the liquors which Thomas and Kelly and Gustavson and Widen had employed. That is, the amount of sodium sulfate increased more rapidly than did the free acid of the liquor. As a result, the hide substance was thus subjected to a shrinking action and this shrunken condition was presumed to impede the diffusion of the chrome liquor into the hide substance, thus explaining the drop in chrome fixation. The experiments of Küntzel, Kinzer and Stiasny, dealing with the neutral salt effect in chrome tanning, have been described and discussed on pages 462 to 463. The more recent studies of McLaughlin and Adams on the chrome fixation inhibiting effect of sodium sulfate are described in the same section.

In 1934, McLaughlin, Cameron and Adams[50] studied chrome fixation as a function of liquor concentration. They employed the skin squares already described and also whole pickled calf skins. The tanning materials used were basic chrome sulfate (both sodium sulfate-free and that containing the sodium sulfate formed by reducing sodium bichromate with glucose), chrome alum and basic chrome chloride. Since the results of these various experiments were essentially similar as to the function of chrome concentration, we shall describe only those in which 25 grams of skin squares were tanned in 250 ml of the various chrome sulfate liquors for 48 hours at 90° F and with constant agitation. At the end of tanning, the specimens were washed for 48 hours with running water and were dried and analyzed. The results are shown in Table 176, where all percentages are based on hide substance.

Table 176

2.8% Basic Liquor		17.5% Basic Liquor	
Cr_2O_3 given (%)	Cr_2O_3 fixed (%)	Cr_2O_3 given (%)	Cr_2O_3 fixed (%)
4.22	3.82	6.36	5.21
6.32	4.52	8.45	6.00
8.45	4.87	10.58	6.49
10.55	5.12	12.67	6.94
12.63	5.42	14.81	7.23
14.77	5.62	16.90	7.40
16.85	5.66	19.04	7.76
18.95	5.75	21.12	7.90
21.10	5.82	23.28	8.22

36.7% Basic Liquor		46.0% Basic Liquor	
Cr_2O_3 given (%)	Cr_2O_3 fixed (%)	Cr_2O_3 given (%)	Cr_2O_3 fixed (%)
9.30	7.98	9.30	8.75
10.36	8.58	10.33	9.61
11.39	9.22	11.35	9.76
12.41	9.64	12.37	10.75
13.44	9.83	13.35	11.50
14.50	10.16	14.47	11.75
16.55	10.58	16.51	12.45
18.65	10.98	18.56	13.29
20.70	11.32	20.66	13.65
22.80	11.61	22.70	13.86
		24.76	13.99

These authors then pointed out that if the fixed chrome values noted in the table were plotted as ordinates and the unfixed chrome values as abscissae, the typically parabolic curves shown in Figure 144 were obtained, and that

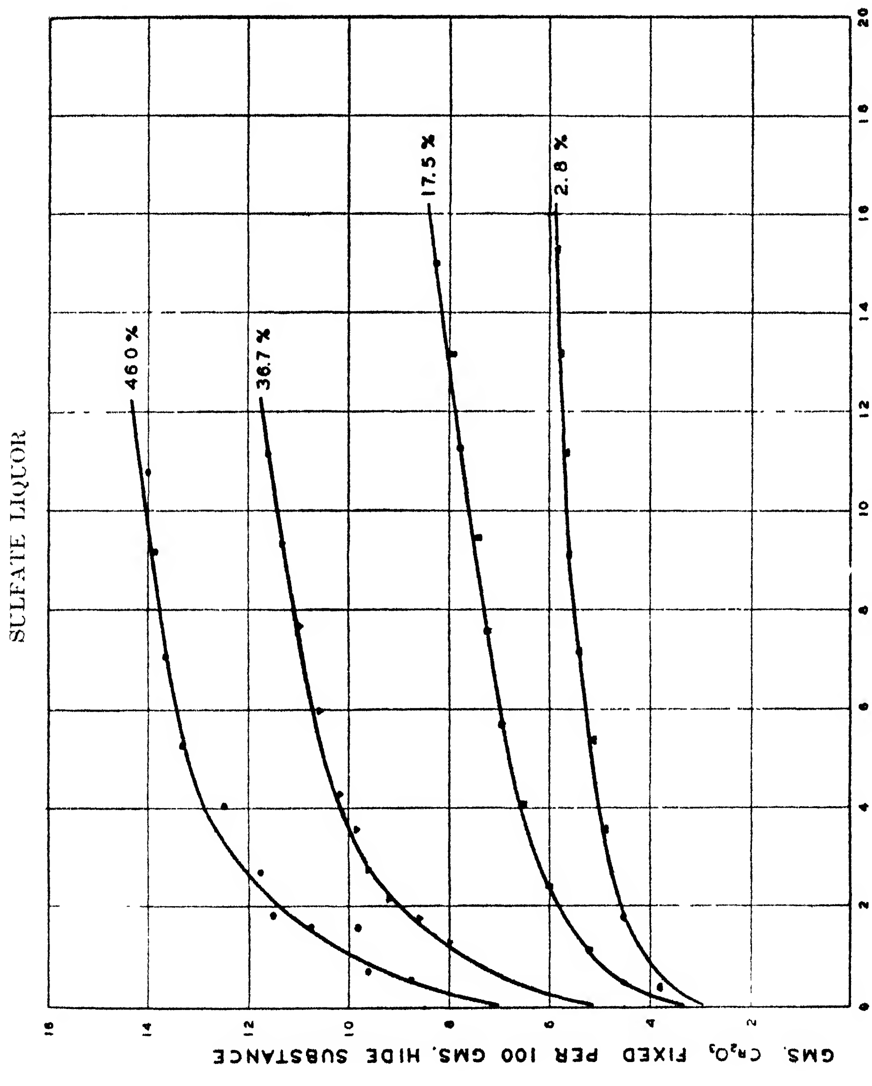


Figure 144. Dehydrated Skin Tanned with Glucose Reduced Basic Chrome Sulfate Liquor of the Basicities Indicated.

plotting the logarithms of these values yielded the straight lines shown in Figure 145. It will be noted from Table 176 that the highest Cr_2O_3 concentration employed was 24.76 grams per liter, and it may be objected that the straight-line function shown may not apply in the case of higher concentrations.

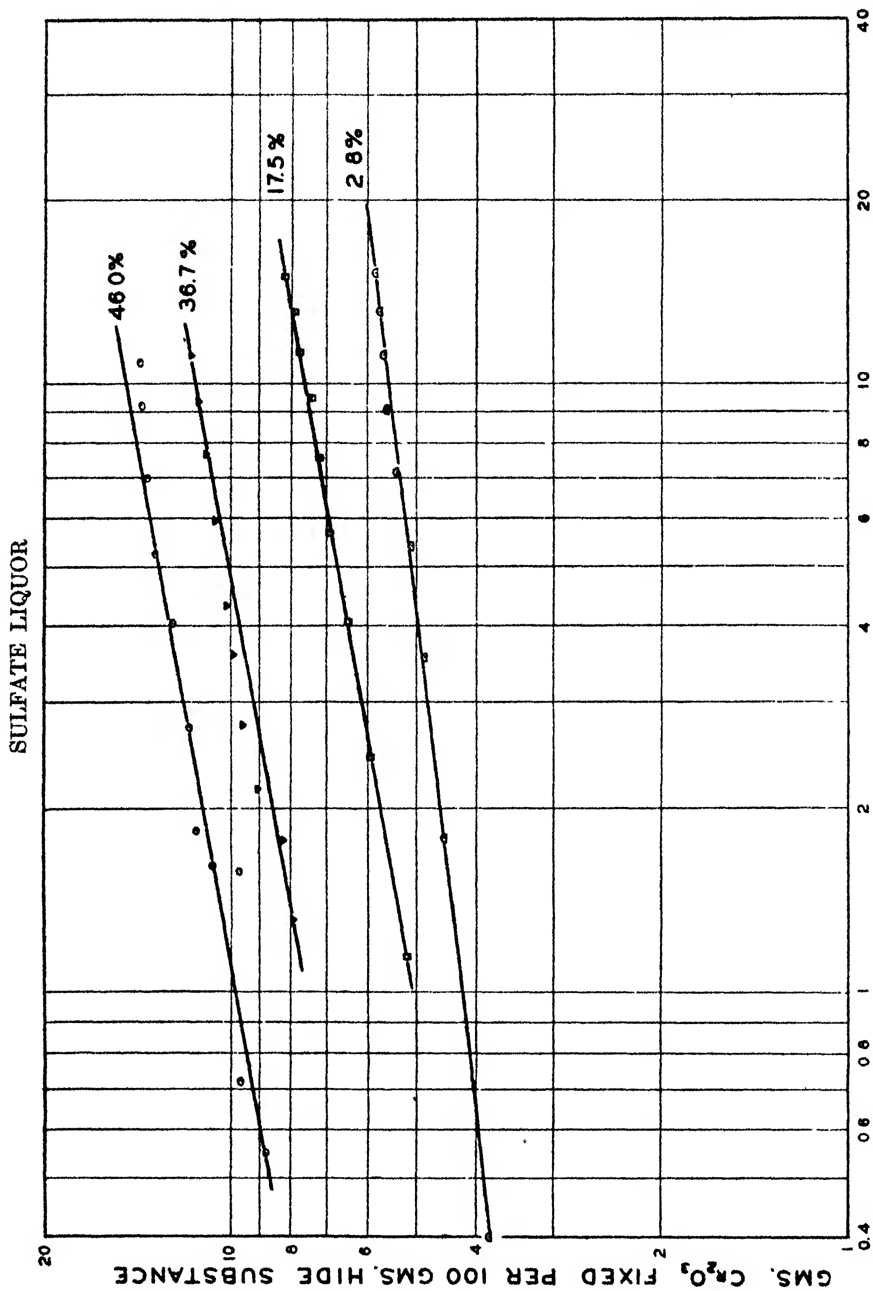


Figure 145. Dehydrated Skin Tanned with Glucose Reduced Basic Chrome Sulfate Liquor of the Basicities Indicated.

We have therefore recalculated the values of the very extensive concentration experiments of Gustavson and Widen, referred to above. Their fixation results also give straight lines in every case, that is, up to the point of decreasing chrome fixations, *i.e.*, up to 75.00 grams Cr_2O_3 per liter of a 35.0 per cent basic chrome sulfate.

In order to ascertain whether any drop in chrome fixation occurs as a function of liquor concentration when a neutral salt and organic acid free liquor is employed, McLaughlin and Adams[51] recently tanned skin squares with liquors ranging from 10.0 to 150.0 grams Cr_2O_3 per liter. The description of the preparation of the 42.5 per cent basic chrome sulfate liquor which was employed is given on page 466. Thirty grams of skin squares were tanned in 300 ml of the various concentrations for 48 hours at 90° F and with constant agitation at 19 rpm. Tanning equilibrium was reached in this period. They were then removed and divided into three 10.0-gram portions, which were treated as follows. One portion was pressed twice at 5000 pounds, the second portion was washed in running water at 70° F for 48 hours, and the third portion was pressed twice at 5000 pounds and then immediately washed in running water for 48 hours at 70° F. All the samples were then air-dried at room temperature and were ground and analyzed. The results are shown in Table 177, where all percentages are based on hide substance.

Table 177

Cr_2O_3 Given (%)	Liquor Basicity	Per cent Cr_2O_3 Fixed: Pressed	Washed	Pressed and Washed	Basicity of Pressed Leather
10.00	42.50	9 36	9.46	9.08	41.70
15.00	42.50	12 06	12.18	12.10	43.10
20.00	42 50	14.26	14.45	14.25	43.30
25.00	42.50	15.62	15.74	15 30	42.90
30.00	42 50	16.99	17.10	16.00	42.80
35 00	42.50	18.70	18.10	17.09	42.80
40.00	42.50	19.48	18.36	17.80	41.70
45.00	42.50	20.40	19.36	17.87	44.00
50.00	42 50	21.27	19.45	18.45	41.80
60.00	42.50	22.26	19.40	18.66	40.80
70.00	42.50	22.90	19.28		
80.00	42.50	23.83	19.45		
90.00	42 50		20 00		
100.00	42 50	25 55			
150.00	42.50	25.80	19.83	19 00	

The results shown in Table 177 are illustrated in Figure 146. It will be noted from the table and figure that when hide substance is tanned to equilibrium with a liquor which contains no neutral salts or organic acid there are no drops in the fixation curves. Chrome fixations up to and including 25.00 per cent Cr_2O_3 given show practically identical values, whether uncombined matters are removed by pressing, by washing or by pressing and washing. But when 30.00 per cent or more chrome is given, fictitious fixation values

result; that is, pressing at 5000 pounds does not completely remove the unfixed chrome, and washing causes the unfixed strong liquor to hydrolyze and to deposit chrome. In such cases, it is therefore best to first press the leather and then wash it. When this latter procedure is followed, we note that the fixation curve flattens out, starting at 50.00 per cent Cr_2O_3 given. McLaughlin and Adams have explained this phenomenon as follows. The collagen must remove acid from the diffused chrome liquor until the chrome reaches a basicity of 66.7 per cent, at which point insoluble chrome is deposited and tannage results. Bringing 1.00 gram Cr_2O_3 from its original (liquor)

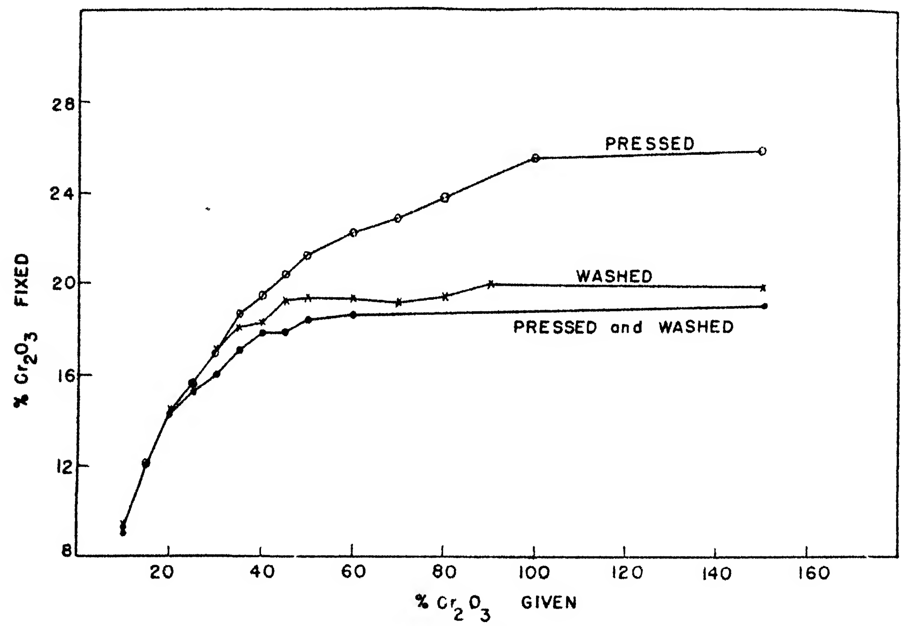


Figure 146

basicity of 42.5 to 66.7 per cent basic requires the removal, by the collagen, of 0.46 gram of acid sulfate. Multiplying 0.46 by 18.50 (grams Cr_2O_3 fixed when the pressed and washed curve flattens out) equals 8.51 grams of acid sulfate to be fixed by 100.00 grams of collagen. The approximate maximum acid-combining capacity of the collagen has thus been reached; the collagen is unable to fix any more acid and, consequently, cannot fix more chrome.

Referring to Table 177, it is seen that the basicity of the pressed leather is very approximately that of the chrome liquor in which it was tanned. When the logarithms of fixed Cr_2O_3 are plotted against the logarithms of unfixed Cr_2O_3, straight lines are derived for the pressing experiment up to and including 100.00 per cent Cr_2O_3 given, and up to 50.00 per cent given in the

case of both the washed and the pressed and washed experiments—in other words, up to the point where the fixation curve flattens out in all three cases. The derived straight lines do not explain the nature of the reaction involved, but they illustrate its course.

Effect of Neutral Salts in Chrome Tanning

We have noted in Chapter 11 that pickling consists of treating the hide or skin with a mixed solution of acid and salt prior to chrome tanning. The importance of the kind and amount of pickling salt is very great; the salt may have far-reaching effects on the skin and the chrome liquor added, as well as on the reaction between them and on the characteristics of the resulting leather. Or, salts added to the chrome liquor before it enters the tan drum also produce large effects. We shall deal more particularly in this section with the manner in which salts affect the composition and tanning characteristics of chrome compounds. Since this subject is both complex and far-reaching, it will be well to discuss inorganic and organic salts separately.

Inorganic Salts. The inorganic salts used in pickling are usually confined to sodium chloride and sodium sulfate, and the latter is sometimes added to the chrome liquor. Also to be considered is the effect of the sodium chloride formed in the case of basic chrome chlorides, and the sodium sulfate formed in the case of basic chrome sulfates, when chrome liquors are made with sodium bichromate and acid, including liquors reduced with sulfur dioxide. In all these cases tanning is actually performed with a mixture of chrome compounds, acid and neutral salts; such mixtures may include both sodium chloride and sulfate when a pickle of sulfuric acid and sodium chloride is followed with either basic chrome chloride or basic chrome sulfate. These procedures of pickling have been in use ever since the advent of chrome tanning, but it is only within recent years that the salt effect has been experimentally studied.

In 1920, Wilson and Gallun[106] made up a 35 per cent basic chrome sulfate liquor of a dry commercial extract from which they prepared a series of solutions of varying concentrations of different salts, with all solutions containing a final concentration of 17 grams Cr_2O_3 per liter. They then placed 16-sq in specimens of pickled calf skin in bottles and added to each 200 ml of the chrome/salt solution and tanned them for 24 hours at room temperature, with occasional agitation. After tanning, the leather specimens were washed free of uncombined matters with water. Part of each washed specimen was boiled in water for five minutes and its area shrinkage noted; the remainder of each washed specimen was analyzed for fixed Cr_2O_3. The per cent chrome fixations on hide substance are shown in Table 178.

In another experiment these authors studied the effect of adding sodium sulfate to the same chrome liquor but with a final Cr_2O_3 concentration of 10

Table 178

Moles salt added per liter of chrome solution	Ammonium Chloride	Sodium Chloride	Lithium Chloride	Magnesium Chloride
None	7.35	7.35	7.35	7.35
0.50	5.53	5.70	6.78	5.22
1.00	4.42	4.90	5.72	3.96
1.50	3.25	4.45	4.80	3.41
2.00	2.95	2.97	3.91	3.54
3.00	2.57	2.73	3.98	4.96

grams per liter and three days' tanning; they reported 10.09 per cent Cr_2O_3 fixed by the control, 5.50 in the presence of 0.50 mole sulfate, 4.07 with 1.00 mole and 3.57 with 1.50 moles. They noted that all specimens shrank during boiling, with the exception of the controls. We cannot draw too many conclusions from these experiments, since pickled skin was employed and the actual or potential effect of the various chlorides added may have been partially masked by the effect of the sodium chloride used in pickling. It must also be noted that the tanning experiments were not run to equilibrium and that tremendous excesses of salts were employed in relation to the amount of Cr_2O_3 present in the tan solutions. But their experiments did indicate the importance of the salt effect and they stimulated further studies.

In 1920 Wilson[107] stated that, whereas pickled calf skin tanned for less than two days in the chrome liquor described above did not shrink in boiling water, it did shrink if the chrome liquor was made to contain 120 grams NaCl per liter, even though tannage was extended to seven days. He found a 50 per cent increase in hydrogen ion concentration when the chrome liquor contained salt as noted. Wilson then investigated the effect of added salts upon the precipitation figure of a commercial chrome sulfate (as described above, and which contained the Na_2SO_4 formed in its preparation). This was done by slowly adding with agitation 0.1N NaOH to 10 ml of a filtered chrome solution containing 17 grams Cr_2O_3 per liter until a permanent turbidity due to precipitation of chrome appeared. In this way it was found that the control solution required 3.7 ml of alkali; but when 0.04 gram molecule of NaCl was added to another 10-ml portion, 6.8 ml of alkali were required. The procedure described was repeated by adding 0.02 gram molecule of various salts to 10 ml of the chrome solution. As a result, the order of degree of effectiveness in preventing precipitation could be arranged as follows:

$$KCl < NH_4Cl < NaCl < MgCl_2 < MgSO_4 < Na_2SO_4 < (NH_4)_2SO_4.$$

The solution containing potassium chloride required only 4.0 ml alkali, whereas 11.6 ml were required in the case of ammonium sulfate.

Wilson and Gallun had attributed the effect of added chlorides, in part, to their well known hydration capacity: that is, if the added salt removed

water from its role of solvent, the effect would be to increase the concentration of all the chrome liquor constituents, including hydrogen ion. They reasoned that if such concentration of chrome exceeded an optimum for chrome fixation, a decreased chrome fixation should result, as shown in Table 178. Since Thomas and Baldwin[101] had previously shown that addition of chlorides to a pure chrome sulfate solution raised its hydrogen ion concentration and that the addition of sulfate lowered it, Wilson and Gallun suggested the following. They assumed the action of sulfates to be different from that of chlorides; *i.e.*, whereas the chloride effect could be assumed to be mainly one of hydration, the sulfate effect was primarily one of sulfate reaction with the original chrome compound, whereby new compounds with lowered tanning capacity were formed.

To throw further light upon this complicated problem, Thomas and Foster[102] studied the effect of adding sodium chloride, sodium sulfate, and sucrose to a 33.3 per cent basic chrome sulfate prepared by reducing sodium bichromate with sulfur dioxide. The non-electrolyte sucrose was chosen because of its well known hydration capacity in aqueous solution, and it was believed that if chrome fixations were lowered because of the hydration of added chlorides, fixations should also be decreased by the presence of sucrose. Five-gram portions of anhydrous hide powder were tanned with agitation for 48 hours at room temperature with 200 ml of chrome liquor containing 3.0, 15.5 and 100.0 grams Cr_2O_3 per liter, respectively. The tanned powders were then washed free of uncombined matters, dried, and analyzed. The series which contained added sodium chloride, ranging from 0.5 to 4.0 molar, all showed decreased chrome fixations compared with the control and in the case of all three chrome concentrations; the pH values of the exhaust tan liquors were determined (in the case of the 15.5 g.p.l. concentration) and were found to decrease from 2.90 for the control to 2.20 in the case of the 4.0 molar NaCl addition.

The chrome fixation in the series to which sodium sulfate was added also showed lowered chrome fixation in all cases; but in this case the pH value of the exhaust tan liquors increased from the control of 2.85 to 3.01 in presence of three moles of added sodium sulfate. But there was no change in chrome fixation where one, two, and three moles of sucrose had been added to the chrome liquor which contained 15.5 grams Cr_2O_3 per liter; when four moles of sucrose were present a very slight chrome fixation decrease was found. No pH values are reported for the exhaust chrome liquors containing added sucrose. In interpreting their experimental results, Thomas and Foster suggested that the lowered chrome fixations they found were not due to hydration phenomena but to the fact that both sodium salts may have formed more complex chromium compounds of lessened reactivity with hide substance. We would emphasize that the neutral salt studies of both Wilson and

Thomas and their collaborators were made in what may be termed the infancy of chrome-tanning studies, and that they pointed the way to future investigations.

In 1926 Gustavson[21] studied the effect of treating standard hide powder with various neutral salts before chrome tanning, and in connection with similar studies relating to vegetable tanning described in Chapter 17. One hundred-gram portions of hide powder were soaked for 14 days in molar solutions of the various salts in stoppered bottles covered with toluene, presumably at room temperature. The soaked powders were then freed of salt by washing and the amount of dissolved hide substance was determined. The washed powders were then dehydrated with 95 per cent alcohol and air-dried. Portions of the dry powders thus prepared, ranging from 2 to 5 grams in the several experiments, were tanned for 48 hours at room temperature with 200 ml of chrome solution under constant agitation: uncombined matter was then washed out and the tanned powders were dried and analyzed for fixed Cr_2O_3.

The results may be summarized as follows. (1) When a 59 4 per cent basic chrome sulfate (diluted to 15.4 grams Cr_2O_3 per liter immediately before use), which showed both cathodic and anodic migration, was employed, the chrome fixations varied greatly as a function of previous salt treatment. Thus, whereas the control showed 23.68 per cent Cr_2O_3 fixed on hide substance basis, the powder treated with sodium chloride fixed 24.22 per cent, that with sodium sulfate 18.24 per cent and that with calcium chloride 29.84 per cent. (2) When a 54.2 per cent basic chrome sulfate liquor which showed "considerable anodic migration" was used immediately after dilution to 11.8 grams Cr_2O_3 per liter, the control showed 18.00 per cent fixed Cr_2O_3; the sodium chloride, sodium sulfate and calcium chloride treatments showed 18.23, 15.14 and 22.46 per cent fixed Cr_2O_3, respectively. But it is important to note that when the same 54.2 per cent basic liquor was allowed to age four weeks before dilution for use (and when it showed only cathodic migration), there was no appreciable difference in the degree of chrome fixation. Nor were any fixation differences found when a 37.0 per cent basic sulfate liquor showing cathodic migration only was employed; and the same finding held true for an 8.0 per cent basic sulfate which showed only cathodic migration. (3) When a 30.2 per cent basic chrome chloride, which showed cathodic migration only, was employed, no fixation differences were found. (4) Considerable chrome fixation differences were, however, found when anionic chromiates were employed. The chrome compound used was a sodium sulfito-chromiate showing anodic migration only and was diluted to 11.3 grams Cr_2O_3 per liter. In this case the control fixed 20.83 per cent Cr_2O_3, the sodium chloride-treated powder 21.32, the sodium sulfate-treated 16.84 and the calcium chloride-treated 30.62 per cent.

Gustavson interpreted the results just described as due to the effect of the

various salts upon the secondary valence forces of the collagen. He concluded that primary valence forces were involved in the fixation of cationic chrome compounds, whereas secondary valences were largely responsible for anionic chrome fixation. Stiasny[76] has confirmed Gustavson's findings as to the lack of neutral salt effect upon chrome fixation in the case of cationic compounds. He finds a slight effect in the case of anionic compounds but states that such chrome fixation differences are apparent only with unbated hide pieces. Stiasny states that the character of liming is of much greater importance in determining the degree of chrome fixation than is the neutral salt effect.

Gustavson[22] then studied the effects of adding neutral salts to the chrome liquor itself. He prepared a series of chrome chloride liquors of varying basicities by adding dilute sodium hydroxide to a solution of C. P. chrome chloride, $CrCl_3 \cdot 4H_2O$. The liquors were then boiled and were aged four weeks. Various concentrations of chrome solutions were then prepared in a range of from 1.90 to 38.20 grams Cr_2O_3 per liter. For each chrome concentration studied there were prepared aliquots containing an increasing concentration of added sodium chloride; that is, all the solutions of a given series contained a constant amount of Cr_2O_3, but were 0.25, 0.50, 1.00, 2.00, 3.00, 4.00 and 5.00 molar with respect to sodium chloride. The precipitation figure of the various mixtures just described was determined by slow additions of 0 1*N* NaOH, with agitation, to 5.0- or 10.0-ml portions of the chrome/salt solution until a permanent turbidity was reached: this procedure was followed immediately after making up the solutions and again after they had stood for six weeks.

It was thus found that, regardless of either the chrome concentration or its basicity, the presence of added sodium chloride decreased the precipitation figure in all cases. Additions of small amounts of sodium chloride caused a slight rise in the pH value of the chrome solutions, but larger amounts of salt caused a marked decrease compared with that of the salt-free control. No direct relation was found to exist between the hydrogen ion concentration and the precipitation figure of the various solutions. Gustavson pointed out that the precipitations noted are not a function of hydrogen ion concentration. He stated: "The progress of the agglomeration process, which finally leads to precipitation, is measured by the degree of condensation of the chrome compound, which is in its turn controlled by the nature of the internal sphere, primarily in regard to chlorine content. The per cent acidity at which precipitation occurs is proportional to the ratio of chlorine to chromium in the complex cation." It must be kept in mind, of course, that experimental results in which great salt concentrations are present may not necessarily be applicable to tannery processes, where much less salt is used.

Stiasny and Ziegler[81] also studied the effect of adding sodium chloride to chrome chloride solutions. This was done by adding portions of sodium

hydroxide or carbonate at one-minute intervals to 25.0 ml of different chrome chloride solutions containing one gram of Cr per liter. Since they found that the visual observation method employed by Wilson and by Gustavson did not yield highly accurate precipitation figures, these authors employed a nephelometer, that is, a Zeiss step photometer with a cloudiness scale. By this means they studied the effect of adding sodium chloride to both a zero basic and a 33.3 per cent basic chrome chloride, both without heating and after boiling for three hours. Their findings may be summarized as follows. When the chrome solutions contained sodium chloride in the proportion of one mole of salt per mole of Cr, little or no change occurred in the precipitation figure; and even when the salt concentration was increased to the enormous proportion of fifty moles per mole of Cr the change in precipitation figure was not very great. In commenting upon these results, Stiasny[76] states that the effect of added sodium chloride upon the precipitation figure of pure chrome chlorides of moderate basicity and but slight olation is not great; but he points out that the effects may be different in the case of technical chrome chloride liquors, where other influencing substances may be present.

Gustavson[22] next studied the effect on chrome fixation of the addition of chlorides to solutions of C.P. chromic chloride which were brought to various basicities by the addition of sodium hydroxide. When these solutions had reached equilibrium they were diluted with salt solutions in such manner that there was a constant chrome content throughout a given series and an increasing salt content. The chrome concentrations thus studied ranged from 6.7 to 100.0 grams Cr_2O_3 per liter, the basicities from 16.7 to 75.5 per cent, and the salt concentrations present in the chrome solutions from 0.25 to 5.0 molar. Tanning was performed by agitating for 48 hours at room temperature approximately 5 grams of hide powder with 200 ml of chrome liquor. The tanned powder was washed free of uncombined chrome, dried and analyzed. The findings may be summarized as follows Moderately concentrated solutions of chrome chloride show an increased Cr_2O_3 fixation in the presence of added neutral chlorides, although their hydrogen ion concentration is increased. In more concentrated chrome solutions the fixation is decreased by added chlorides. Changes are brought about in the degree of dispersion of extremely basic chrome chloride solutions when neutral salts are added. The pH value of all solutions studied was progressively lowered as a function of increasing salt concentration. Gustavson suggests the following interpretation of these experiments: "The main finding of this investigation is that the degree of chrome fixation is not regulated by the hydrogen ion concentration, but largely by the constitution of the chromic salt."

Gustavson[18] also studied the effect of adding sodium sulfate to a 37.0 per cent basic chrome sulfate liquor. These experiments, as well as the chloride experiments described above, were the first adequate studies in which chrome

fixations, etc. were observed with pure materials, and in which the influence of similar ions was determined. The 37.0 per cent basic liquor was made by reducing a mixture of C.P. sodium bichromate and sulfuric acid with sucrose. The basicity value obtained by titration was the same as the theoretical, indicating the absence of organic acid in the completed liquor. Five-gram portions of hide powder were tanned for 48 hours with 200 ml of this liquor having a concentration of 22.6 grams Cr_2O_3 per liter, and to which was added sodium sulfate as shown in Table 179. The uncombined chrome was removed by washing at the end of tanning and the tanned powders were dried and analyzed.

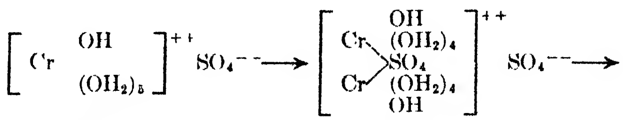
Table 179

Moles of Na_2SO_4 present in chrome solutions	Electrical Migration	Cr_2O_3 combined with collagen (%)
0.00	Cathodic	12.14
0.25	Cathodic	8.93
0.50	Cathodic and slight anodic	7.39
1.00	Marked anodic	4.43

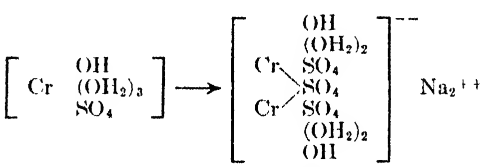
Gustavson felt that the changes induced in the composition and electrical behavior of the chrome complex by the addition of sodium sulfate are of great importance in determining its tanning behavior. But it must be noted that, whereas the addition of sufficient sodium sulfate to render the chrome solution 0.25 molar (that is, 1.57 parts added Na_2SO_4 to each 1.00 part Cr_2O_3) reduced chrome fixation some 26 per cent, it had no effect on the electrical migration of the chrome. Twice this amount of added sulfate had only slight effect upon migration, and four times this amount was required to bring about marked anodic migration.

Stiasny and Ziegler[81] have studied the effect of adding sodium sulfate to chrome sulfate solutions on their precipitation figures. This was done by means of the nephelometer method already described. They employed a chrome concentration of 1.46 grams of Cr_2O_3 per liter and observed the effect of adding 0.5 mole Na_2SO_4 per 1.0 mole and also 25.0 moles of Cr—in other words, 0.93 part Na_2SO_4 per part Cr_2O_3 and 16.5 parts in the case of the 25.0 moles. (The ordinary chrome liquor prepared from sodium bichromate contains 0.93 part Na_2SO_4 per 1.00 part Cr_2O_3.) They found practically no change in precipitation figures when 0.5 mole Na_2SO_4 was added to a cold hexa-aquo-chromi-sulfate (zero basicity) and only a slight increase with 25.0 moles. Small increases were found if the chrome solutions were boiled under reflux for three hours before addition of sulfate; these were attributed to the effect of hydrolysis and olation. A very slight increase in precipitation figure was found when 0.5 mole of sulfate was added to an unheated 33.0 per cent basic liquor, with considerable increase for 25.0 moles added. When sodium carbonate was employed for raising the basicity of the chrome solution from

zero to 33.0 per cent instead of sodium hydroxide, there were greater increases in precipitation figures in the presence of 25.0 moles of added sodium sulfate. Stiasny and Ziegler believe the precipitation figure increases found with the high sulfate concentrations to be a result of sulfate radicals being pushed into the chrome complex, whereby it becomes more alkali-stable. This change may be pictured as follows:



2 positive charges per 1 Cr — 1 positive charge per 1 Cr



Neutral complex — 1 negative charge per 1 Cr

Küntzel, Kinzer and Stiasny[43] have investigated the possible effect upon chrome fixation of the sodium sulfate which is normally present in basic sulfate liquors and which is one of the reaction products of the reduction of sodium bichromate. They were interested in determining whether such salt could be responsible for the findings of Gustavson, of Wilson and of Thomas and Foster that the amount of chrome fixed by hide substance from basic chrome sulfate liquors decreased with increasing strength of chrome liquor after a certain optimum concentration was exceeded. Küntzel, Kinzer and Stiasny reasoned that as basic chrome liquors became more concentrated the proportion of free acid present to neutral salt decreases; the acid does not increase in the same ratio as the neutral salt. Because of this, they visualized the possibility of a pickling action (diminution of swelling and hydration) upon the hide substance by the neutral salt. If this were true, the diffusion of chrome solution into the collagen would be hindered and chrome fixation would be reduced. They therefore tanned hide powder with increasing concentrations (up to 463 per cent Cr_2O_3 on hide substance basis) of a 40.5 per cent basic chrome sulfate liquor. (This liquor was so prepared that it contained no neutral salts; it was made by the action of hydrogen peroxide on chromic acid and sulfuric acid.) Duplicate tannages were then performed with the same chrome liquor, to which had been added increasing concentrations of sodium sulfate: 0.55 and 1.00 mole per mole of Cr. The chrome-fixation curve for the salt-free liquor shows no drop as a function of chrome concentration, rising continuously to 10.95 per cent fixed Cr_2O_3 at the highest

concentration. The series of liquors containing 0.55 mole Na_2SO_4 per mole of Cr show 9.50 per cent fixed Cr_2O_3 at the highest chrome concentration, and that containing 1.0 mole 5.50 per cent. These experiments are unfortunately not very convincing, since they were not run to equilibrium; this is apparent from the fact that a maximum of only 10.95 per cent Cr_2O_3 was fixed, as noted. Had they been run to equilibrium a much greater fixation, at least 17.00 per cent, would have resulted. In a later communication, Küntzel and Riess[44] restate their conception of the experiments just discussed; that is, that if a chrome liquor contains too great a concentration of a pickling, neutral salt the collagen will be dehydrated. When such dehydration occurs, the inter- and intra-micellar spaces are diminished; this obstructs the entrance of chromium aggregates and so reduces chrome fixation.

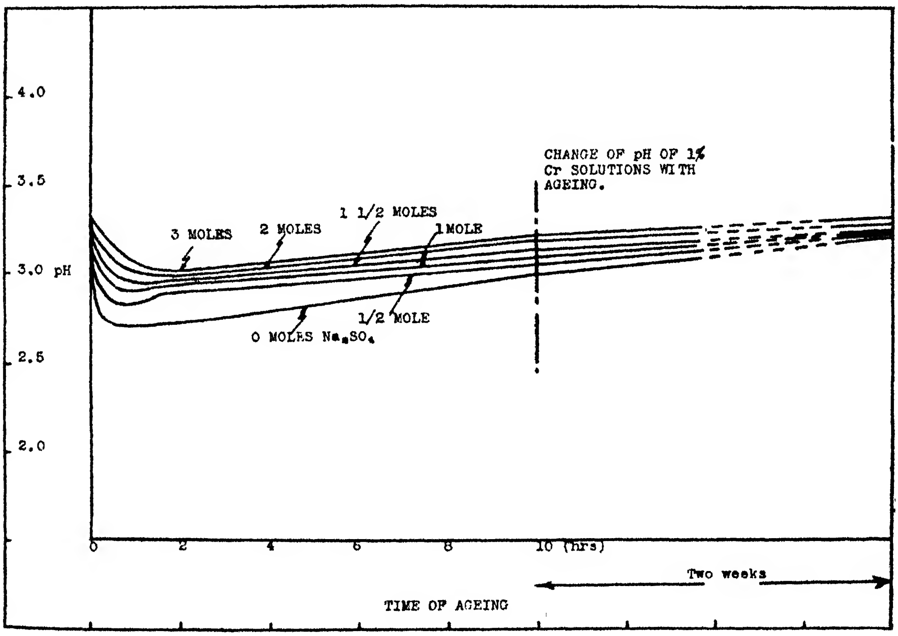


Figure 147

Serfass and Theis[73] have studied the effect of adding sodium sulfate to a 36.0 per cent basic chrome sulfate which they describe as a chemically pure compound. Portions of this dry compound were weighed and to them was added sodium sulfate to give a series of liquors containing 0.50, 1.00, 2.00 and 3.00 moles sodium sulfate per mole of chromium, respectively. These mixtures, as well as the control, containing no added salt, were dissolved in sufficient water to produce an approximately 10.0 per cent chromium solution

after boiling for 10 minutes under reflux. After cooling, the several solutions were accurately diluted to a concentration of 5.0 per cent chromium, were boiled under reflux for 30 minutes, and then rapidly cooled and made up to mark. The solutions were diluted immediately after cooling to a concentration of one per cent chromium, the constants of which were determined immediately and, also, after they had aged up to two weeks at room temperature. Changes in pH values, as a function of aging time and added sodium sulfate content, determined by a glass electrode, are shown in Figure 147. The figure shows that, as had been noted by other workers, the addition of sodium sulfate increases the pH value of the basic chrome sulfate solution; that aging at first decreases the pH value; and that this value soon returns to that of the unaged solutions.

The effect of added sodium sulfate upon the amount of complexly bound sulfate of the chrome solution described was determined; the results are shown in Table 180, which indicates that, whereas the addition of appreciable

Table 180

Moles Na_2SO_4 added per mole Cr	---Moles of Complexly bound SO_4 per mole of Cr---	
	15 min. after cooling	6 hours after cooling
0.0	0.527	0.464
0.5	0.563	0.452
1.0	0.710	0.435
1.5	0 920	0.455
2.0	1.084	0.405
3.0	1.435	0.473

quantities of sodium sulfate to a one per cent solution of a 36.0 per cent basic chrome sulfate greatly affects the amount of complexly held sulfate of an unaged solution, such effects are absent after the solution has aged for six hours.

An attempt was made to determine the relative molecular weights of the chromium compounds in the presence of added sodium sulfate and in its absence. (The method employed for this was the diffusion procedure suggested by Riess and Barth, described on page 524.) The results are shown in Figure 148. The figure shows that when sodium sulfate was added to a one per cent Cr solution immediately after cooling the boiled solution, the presence of 0.5 mole salt presumably decreased the molecular weight of the chrome compound. Further increases in salt addition caused a slight rise in molecular value. If the chromium-sodium sulfate mixtures were allowed to stand for one hour before diffusion, the curve was essentially of the same shape as that of the unaged solutions, but with slightly lower values, until the solution became 3.0 molar; but after three hours' aging all the molecular weights increased.

Theis[90] tanned hide powder with basic chrome sulfate liquors containing

a constant amount of Cr_2O_3 and increasing concentrations of added sodium sulfate, so that the various chrome solutions were from 0.25 to 3.00 molar in respect to the added salt. After tanning, washing and drying, the samples were analyzed for fixed Cr_2O_3. This value was found to decrease as a straight-line function from 7.00 per cent for the control to 6.00 per cent in the presence

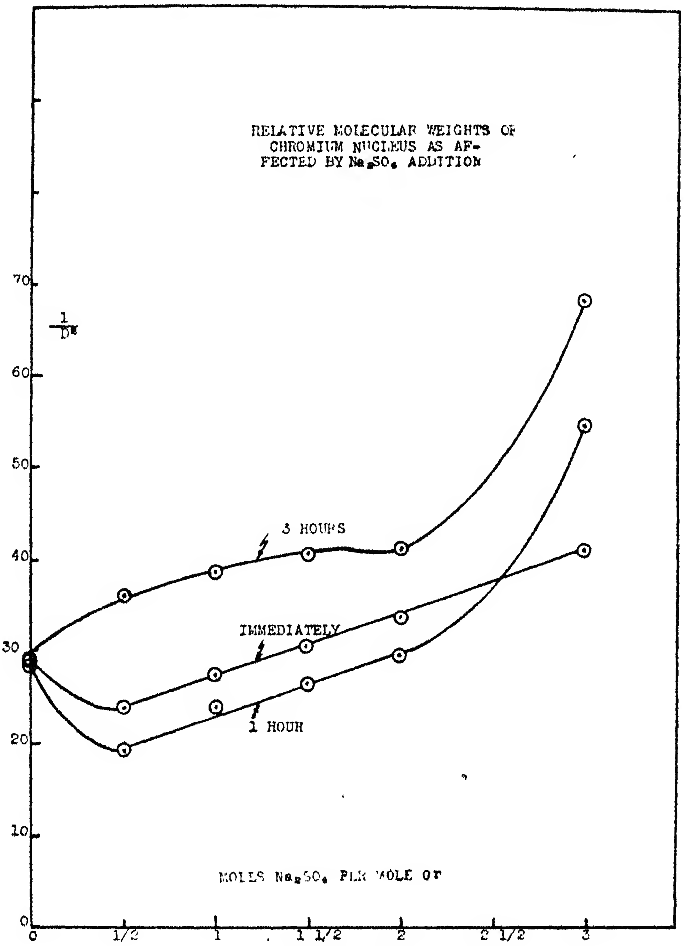


Figure 148

of 3.00 moles added sodium sulfate. Theis considered the decreased fixations noted to be probably a function of sulfate penetration into the chrome complex, thereby decreasing its reactivity with hide substance.

Theis and Meerbott[91] repeated the hide powder experiments described above, with 5 grams of powder, 100 ml of a 1.0 per cent Cr_2O_3 33 per cent basic

sulfate liquor, tanned for 48 hours at 25° with constant agitation. The various chrome solutions of this series contained added sodium sulfate, as noted. The results are shown in Table 181, which gives the analysis of the tanned powders after they had been washed free of unfixed chrome, salt and acid (as well as of part of the combined acid), by treatment with 5 liters of distilled water at 75° over a two-hour period, after which they were dried, reground and analyzed. The figures given in the table are based upon hide substance.

Table 181

Moles salt added per gram atom Cr	pH of exhaust liquors	Cr_2O_3 (%)	SO_4 (%)	Cr (%)	Overall Leather Basicity (%)
.....	3.10	7 95	7 62	5.44	39.3
0.0625	3 08	7.73	7.59	5.28	37.8
0.1250	3.10	7.75	7.72	5.30	36.8
0.2500	3.12	7.73	7 55	5.28	38 2
0.3750	3.14	7.65	7.62	5.23	37 0
0.5000	3.14	7 60	7.52	5.20	37 4
0.7500	3.18	7.51	7.55	5.14	36.3
1.0000	3.22	7.39	7.31	5.05	37 3
1.5000	3.23	7.12	7 21	4.87	35 8
2.0000	3 25	6.87	6 97	4 70	35 7

The authors ascribe the decreased chrome fixations with increasing sodium sulfate additions, as shown in the table, to the possible formation of anionic complexes with lessened tanning power.

We have noted that small additions of sodium sulfate to a basic chrome sulfate liquor may decrease its tanning power, even though the precipitation figure and electrical migration of such liquor may remain unchanged and the proportion of its ionically and complexly held acid radicals may be unaffected. In view of these facts, McLaughlin and Adams[52] sought an explanation for the decreased tanning power of basic chrome sulfate liquors containing sodium sulfate. They believed the phenomenon might be directly related to a decreased acid binding by hide substance when sodium sulfate is present in the chrome liquor. If less acid is fixed by the hide substance, less chrome is brought to a basicity of 66.7 per cent, at which point it is deposited in and on the skin fibers. The experiments which follow indicated to these authors that their conception was correct.

McLaughlin and Adams prepared a neutral salt-free basic chrome sulfate liquor of 42.5 per cent basicity by the following means. Chromic acid was reduced with sulfur dioxide, after which it was boiled for three hours and showed no SO_2. After standing one week it was heated and sufficient calcium hydroxide was added to bring its basicity to 42.5 per cent; the solution was then boiled for three hours, was allowed to cool and then stood for two weeks at room temperature. The liquor was then filtered to remove $CaSO_4$, the filtered liquor showing 0.01 per cent lime as CaO. To the filtered liquor were added the amounts of sulfuric acid calculated to produce the

basicities desired. The liquors thus prepared were then analyzed and stood several months at room temperature before use; in this way thoroughly aged liquors, containing no neutral salts, were obtained. Ten-gram portions of the 0.5 x 0.5-inch skin squares, described on page 444, were first wet with distilled water; the requisite amount of sodium sulfate solution was then added, followed by the proper amount of chrome liquor; and the total liquid mixture was then made up to 100 ml. The jars containing the skin and liquor were then continuously agitated at 19 rpm for 48 hours, at 120° F. (It will be noted that this temperature was employed rather than the usual 90° F. This was because experiment showed that tanning equilibrium was not reached in 48 hours at 90° F in the case of the higher sodium sulfate concentrations. By "tanning equilibrium" it is meant that the tanned leather at equilibrium, or completion of tannage, must show essentially the same basicity as that of the chrome liquor employed, *i.e.*, the liquor basicity based upon all acid radicals present.)

After tanning, the squares were pressed twice in a hydraulic press at 5000 pounds per square inch to remove all unfixed chrome and acid. The leather was then dried at room temperature, ground, and analyzed; chrome was determined by the perchloric method and total sulfate was determined gravimetrically. Each leather specimen was also analyzed for sodium by means of the Barber and Kolthoff method, and from the results so obtained there was deducted the small amount of physiologic sodium present in the untanned hide substance. In this way it was possible to calculate any neutral sulfate combined with or present in the pressed leather. Deducting such neutral sulfate from the leather's total sulfate yielded the acid sulfate and made possible accurate calculation of basicities.

The results obtained are given in Table 182, where the percentages of materials used, and the analyses of leather, are based on hide substance. We note that in the case of each of the four basicities studied there is a continuous decrease of fixed chrome as the sodium sulfate concentration rises. (In no case was there any apparent shrinking or pickling effect of the sodium sulfate upon the leather squares.) It will be noted that in all cases, regardless of the amount of added salt, the pressed leather shows essentially the same basicity as that of the chrome liquor in which it was tanned. This becomes apparent also when we compare the figures of the last column of the table (which are the theoretical acid sulfate values required to make the leather exactly the same basicity as the liquor) with the acid sulfate values found by analysis of the leather. The pH values of the exhaust chrome liquors rise steadily with increasing sodium sulfate concentration.

The sixth column of the table shows the percentage which the acid sulfate is of the total sulfate present in the chrome liquor. If we plot as abscissae the logarithms of such percentages and plot as ordinates the logarithms of the

percentages of Cr_2O_3 fixed by the leather in each case, the straight lines shown in Figure 26 are derived. These lines would indicate that the lessened chrome fixations noted in the table are directly related to the percentage of acid sulfate in the total sulfate content of the tan liquor. But this finding does not of itself explain the reason for the lowered chrome fixations.

Table 182

% Cr_2O_3 Given	% Acid SO_4	% Na_2SO_4 Given	% Total SO_4	Basicity of Liquor	% of Total SO_4 which is Acid SO_4	% Cr_2O_3 Fixed	% Acid SO_4 Fixed	Basicity of Leather	pH Exhaust Liquor	% Theoretical A SO_4 required to make leather basicity same as liquor
19 34	36 93	0 0	36 93	−0 8	100 0	7 72	15 13	−3 5	1.61	14.75
"	"	5 0	40 31	"	91 6	7 60	14.74	−2 5	1 63	14.52
"	"	10.0	43.69	"	84 5	7 50	14.68	−3 4	1.70	14 32
"	"	20 0	50 45	"	73 2	7 14	13 81	−2 1	1.75	13.64
"	"	30.0	57 21	"	64 5	6 89	13 18	−1 0	1.79	13.16
"	"	40.0	63 97	"	57 7	6 63	12 89	−2 6	1.80	12.66
"	"	50 0	70 73	"	52 2	6 34	12 35	−2 9	1 82	12 11
"	"	60 0	77 49	"	47 7	6 26	12 07	−1 8	1.89	11 95
"	"	80.0	91 01	"	40 6	5 79	11 63	−6 0	1 90	11 06
"	"	100 0	104 53	"	35 3	5.62	11 22	−5 5	1 93	10 74
"	32 27	0 0	32.27	+12 00	100 0	9 17	15 00	13 6	1 90	15 28
"	"	5 0	35 65	"	90 5	8 74	14 49	12 4	1 95	14 56
"	"	10 0	39.03	"	82 7	8 81	14 62	12 7	1 99	14 73
"	"	20 0	45 79	"	70 4	8 73	14 34	13 2	2.02	14 55
"	"	30 0	52 55	"	61 4	8 50	13 96	13 2	2 09	14 17
"	"	40 0	59 31	"	54 4	8 13	13 61	11 6	2 11	13 55
"	"	50 0	66 07	"	48 8	8 01	13.10	13 6	2 11	13 35
"	"	60 0	72 83	"	44 3	7.62	12 70	12 0	2 17	12 70
"	"	80.0	86 35	"	37 4	7 43	12 40	11 9	2 20	12 38
"	"	100 0	99 87	"	32 3	6 97	12 02	9 0	2 21	11 62
"	25.28	0 0	25 28	+31 00	100 0	12 32	15 92	31 8	2 65	16 10
"	"	5 0	28 66	"	88 2	12 10	15 58	32 0	2 71	15 81
"	"	10.0	32 04	"	78 9	11 83	15 39	31 4	2 75	15 46
"	"	20 0	38.80	"	65 1	11.54	14.92	31 7	2 81	15 08
"	"	30.0	45 56	"	55 5	11 05	14.45	31 0	2 87	14 44
"	"	40 0	52 32	"	48 3	10 55	13 90	30 4	2 90	13.79
"	"	50 0	59 08	"	42 8	10.31	13.58	30 5	2 91	13 47
"	"	60 0	65 84	"	38 4	10 07	13 53	20 0	2 95	13 16
"	"	80.0	79.36	"	31 8	9 56	12 75	29 6	2 99	12 49
"	"	100 0	92 88	"	27 2	9.05	12 13	29.2	3 00	11 83
"	21 10	0 0	21 10	+42 5	100 0	14 90	15 39	45 5	2 95	16 22
"	"	5 0	24 48	"	86 2	14.61	15 00	45.8	3.06	15 91
"	"	10 0	27 86	"	75 7	14 23	15 00	44 4	3 11	15 49
"	"	20 0	34 62	"	60 9	13 81	14 82	43 4	3 20	15 04
"	"	30.0	41 38	"	51 0	13.34	14 35	43 2	3 25	14 53
"	"	40 0	48 14	"	43 8	12 92	14 00	42 8	3 30	14 07
"	"	50 0	54 90	"	38 4	12 51	13 22	44 2	3 31	13 62
"	"	60 0	61 66	"	34 3	12 13	13 28	42 2	3 31	13 21
"	"	80.0	75 18	"	28 1	11.18	12 31	41 8	3 31	12 17
"	"	100 0	88 70	"	23 8	10.50	12 04	39 5	3 36	11 43

McLaughlin and Adams then studied the acid fixation by skin squares when a given concentration of sulfuric acid contained increasing concentrations of sodium sulfate. This was done by wetting ten-gram portions of squares with distilled water, adding the requisite amount of acid and sodium sulfate, making the liquid mixture up to 100 ml and agitating for 24 hours at 70° F in a water bath. Experiment showed that equilibrium was reached by this treatment. The specimens were then removed and pressed twice in a hydraulic press, whereby unfixed acid was removed and fixed acid was

retained. The pressed squares were then air-dried, ground and analyzed for fixed acid sulfate. The results are shown in Table 183. The acid sulfate fixed decreases as a function of increasing sodium sulfate concentration, and the pH values of the exhaust acid liquors rise. If we plot the logarithms of the fifth column of the table as abscissae and the logarithms of the acid sulfate fixed as ordinates, the straight lines shown at the right of Figure 26 are obtained; they are of the same general slope as the lines similarly plotted for chrome fixations, shown at the left of this figure.

Table 183

% Acid SO_4 Given	% Na_2SO_4 Given	% Acid SO_4 Fixed	pH Exhaust Solution	% which Acid SO_4 is of total SO_4 in original solution
10.00	0.00	5.86	1.32	100.0
"	5.00	5.72	1.35	74.7
"	10.00	5.51	1.41	59.7
"	20.00	5.02	1.52	42.5
"	30.00	5.15	1.60	33.0
"	40.00	5.01	1.63	27.0
"	50.00	5.01	1.68	22.9
"	60.00	4.98	1.68	19.8
"	80.00	4.87	1.70	15.6
"	100.00	4.84	1 78	12.9
15.00	0.00	6.97	1.00	100.0
"	5.00	6.78	1.01	81.6
"	10 00	6.51	1 08	68.9
"	20 00	6.37	1.11	52.6
"	30.00	6.08	1.20	42.5
"	40.00	6.02	1.30	35.7
"	50.00	5.67	1.33	30.7
"	60.00	5.48	1.35	27.0
"	80 00	5.37	1.40	21.7
"	100 00	5.31	1 40	18.1
20.00	0.00	7.63	0.90	100.0
"	5.00	7.56	0.90	85.6
"	10.00	7.71	0.99	74.7
"	20.00	7.16	0.99	59.7
"	30.00	6.85	1.13	49.6
"	40.00	6.35	1.19	42.5
"	50.00	6.33	1.21	37.2
"	60.00	6.15	1.25	33.0
"	80.00	5.97	1.30	27.0
"	100.00	5.88	1.35	22.8

In view of the findings described above, McLaughlin and Adams conclude that the principal reason for the lowering of chrome fixation in the presence of sodium sulfate in the chrome liquor is the effect of the salt upon sulfuric acid fixation by hide substance. This is especially true in those cases where the amount of added sodium sulfate corresponds to the limit which would be employed in tannery practice. The addition of tremendous quantities of sodium sulfate may, of course, affect the composition of the chrome complex and its electrical migration.

Organic Salts. In 1897 Procter, the great pioneer of leather chemistry, suggested the use of sugars in the reduction of bichromate for one-bath liquors; and shortly thereafter he pointed out that the organic by-products of this reaction probably played an important role in determining the tanning properties of such liquors and the character of leather made therefrom. In 1916, in collaboration with his student, Wilson, Procter[61] showed that the presence of various hydroxy compounds in a one-bath liquor profoundly affects its tanning power. It was demonstrated that whereas the presence of a small amount of hydroxy organic compounds produced a superior leather fullness and softness, an excess prevented complete or satisfactory tannage. Thus was originated the whole subject of "masking compounds," and their effects; a subject which was to prove so fundamental to both leather chemists and tanners.

When we use the term "masking" we usually mean that some compound has been added to a chrome liquor which will change any or all of the following characteristics: the composition of the chrome compound, the pH value of the chrome liquor, the electrical migration of the chrome compound and the liquor's ability to resist precipitation when alkali is added to it. As these changes are induced, the chrome liquor usually behaves differently as a tanning agent: it may cause less or more chrome to be fixed by hide substance compared with the original unmasked liquor, and entirely different leather characteristics may result. The successful employment of masking agents requires careful chemical control and supervision Masking is, of course, brought about by the presence of the organic compounds present in all glucose-reduced liquors, as noted in the discussion of the making of one-bath chrome liquors, where it was stated that the kind and amount of organic substances formed in glucose reduction are governed by the conditions under which such liquors are prepared. Unless great care is exercised to keep uniform the production of glucose liquors from batch to batch, serious differences in the tanning behavior of different lots may be expected. It is for this reason that some tanners may prefer to reduce bichromate with sulfur dioxide and then add the kind and amount of masking compounds desired; in this way uniformity is secured. Masking may also be effected in the absence of added compounds. For example, merely evaporating to dryness a basic chrome sulfate solution may induce great changes in its composition and tanning characteristics; that is, its olation value may be changed, as well as the composition of its complex and its resistance to precipitation by added alkali.

We shall now discuss more particularly the addition of various neutral salts to a chrome-tanning system. These salts may be added to either the pickle solution or the chrome liquor itself. The salts usually employed are sodium oxalate or formate; but other organic salts have been studied and are

included here, as well as sodium sulfite which, though inorganic in constitution, behaves much as do organic salts in masking. We shall not attempt to define the comparative finished leather qualities induced by masking compounds, since such qualities vary with the procedures and methods of different tanneries; but it may be said, in general, that added masking substances may affect the character of the grain and the fullness and smoothness of the finished leather.

In 1923, both Stiasny and Gustavson discussed the addition of organic salts to basic chrome sulfate liquors from the standpoint of Werner's concept, which we have considered in Chapter 14. Gustavson[23] experimentally studied the changes in tanning power of a 33.3 per cent basic chrome sulfate liquor as a function of the addition of increasing amounts of sodium formate or acetate. He found that the addition of small amounts of these salts slightly increased chrome fixation by hide powder or skin, whereas large additions decreased it. He also noted that leather tanned in liquors containing moderate additions of sodium formate possessed superior feel and grain characteristics and hence recommended the addition of formate to the chrome liquors used in practical tanning.

Before proceeding with the description of later experimental studies, it may be well to understand the supposed mechanism of the changes induced in chrome liquors by the addition of masking salts. We noted in Chapter 14 that ions may be arranged in a series of increasing ability to penetrate the chrome complex and that, in so doing, they may displace other complexly held groups. The oxalate ion, for example, possesses great power of complex penetration, and for this reason it is always complexly held. If we add sodium oxalate to a basic chrome sulfate solution, the oxalate ions tend to displace both sulfate and hydroxyl groups from the complex. With increasing concentration of added oxalate the composition of the chrome compound may be drastically changed, as well as its electrical migration. A sharp rise will occur in the pH value of the solution and in its precipitation figure, both of which may be attributed to the displacement of OH groups from the complex, and their emergence into the surrounding solution. The color of the solution usually changes as well. We have dealt with the behavior of the oxalate ion in this paragraph, but the same principles apply to other masking ions, bearing in mind their position in the ion displacement series.

In 1926 Stiasny and Szegö[82] treated a 33.0 per cent basic chrome sulfate liquor with increasing concentrations of sodium sulfite, without heating. The various solutions contained a constant amount of chrome (2.5 grams Cr per liter) and the sulfite concentrations shown in Table 184. The water resistance figures in the last column indicate the per cent of the tanned powder which did not dissolve in distilled water at 100° in 10 hours.

Table 184 illustrates the great changes induced by sodium sulfite in the

character of the chrome compound itself, in its tanning power and in the tanned hide substance. Similar experiments were performed with sodium oxalate as the masking agent and with essentially the same results as when

Table 184

Moles Na_2SO_3 per Mole Cr	Ml 0.1N NaOH to start pptn. in 25 ml	Migration	Per cent Cr in Tanned powder after: 1 hr	4 hrs	24 hrs	Final pH Value	Moles SO_3 per Mole Cr in Leather	Water Resistance (%)
0.00	2 60	Cathodic	0.98	1.45	2.32	2 7	.	86 6
0 25	2.00	Cathodic	0.73	1.23	2 70	3.4	0 23	76.8
0.50	1.15	Cathodic	0.94	1.90	2.92	3.7	0.41	81.6
1.00	2.30	C and A	1.85	2.96	3.60	4.4	0.71	89.1
1.50	∞	Anodic	3.00	3.74	4.56	5.6	0.70	68.4
3.00	∞	Anodic	1.35	1.98	3.24	7.1	0.83	65.8
10.00	∞	Anodic	0.09	0.11	0.16	8.1	. .	24.7

sulfite was employed. These masking changes have been further studied by Stiasny and Szegö, as shown in Tables 185 and 186. Table 185 illustrates the effect of sodium sulfite additions to a hexaquo chrome sulfate solution containing 2.5 grams Cr per liter. The several solutions were boiled 30 minutes under reflux and after cooling were made 33 0 per cent basic by the addition of sodium carbonate, after which the proper amount of sodium sulfite was added. In the case of the oxalate experiments detailed in Table 186, the

Table 185

Moles Na_2SO_3 per mole Cr	Precipitation (ml 0.1N NaOH)	Electrical Migration
0.00	2.60	Cathodic
0.25	2.00	Cathodic
0.50	1.15	C and A
1.00	2.30	Anodic
1.50	∞	Anodic
3.00	∞	Anodic
10.00	∞	Anodic

Table 186

Moles $Na_2C_2O_4$ per mole Cr	Precipitation (ml 0.1N NaOH)	Electrical Migration
0.00	2.60	Cathodic
0 25	1.75	Cathodic
0.50	1.05	C and A
1.00	0.90	Anodic
1.50	∞	Anodic
3 00	∞	Anodic

2.5-gm per liter solution of hexaquo chrome sulfate was boiled for 30 minutes after addition of the required amount of sodium oxalate; the solutions were cooled and were then treated with 0.5 mole sodium carbonate per mole Cr.

Cobb and Hunt[13] added a constant amount of sodium formate to a series of liquors prepared from a 33 per cent basic commercial chrome sulfate extract and then adjusted their pH values to give a pH range of 3.0 to 7.1. This was accomplished by the addition of sodium hydroxide or hydrochloric acid. A similar series was prepared with sodium acetate as masking agent. Pieces of pickled skin were then tanned in the various solutions and the fixed chrome was determined at the completion of the tanning period. They found maximum chrome fixation to occur at around pH 5.0-6.5 in both systems. Their conclusion from these experiments was that if chrome is fixed by collagen by primary valence, there should have been a minimum fixation at the isoelectric point of collagen, *i.e.*, around pH 5.0; but since a maximum fixation actually occurred, they concluded that collagen reacts with chrome by secondary valence forces.

In 1926, Gustavson[24] studied the effect of increasing additions of sodium acetate and sodium formate upon the tanning behavior of a 37.0 per cent basic chrome sulfate liquor. This liquor was prepared from C.P. sodium bichromate and sulfuric acid, with sucrose as a reducing agent. There was no difference between the theoretical and the determined basicity of the completed liquor, which indicated the absence of organic acid. A series of liquors was then prepared containing a constant chrome concentration of 11.3 grams Cr_2O_3 per liter, in which various molarities of salt had been incorporated, as shown in Table 187. Three grams of hide powder were rotated for 48 hours at room temperature with 100 ml of the liquors described. At the end of tanning, the powders were washed with water, dried, and analyzed. These experiments are of special interest because the chrome-acetate series of liquors was aged at room temperature for two years before use and the formate series for eight months. The pH values shown were electrometrically determined.

In 1927, Gustavson[19] tanned hide powder for 48 hours in solutions of basic chrome sulfate which had been made molar in respect to added sodium formate. After tanning, the powders were washed with water; this removed uncombined matter and part of the combined acid. The specimens were then dried and analyzed. In the case of a 59.0 per cent basic sulfate liquor containing formate, 9.46 per cent Cr_2O_3 was fixed on hide substance basis, whereas the control fixed 16.40 per cent; with a 37.0 per cent basic liquor the fixations were 6.84 and 12.06 per cent, respectively. Similar studies employing basic chrome chloride were then made. When a 30.0 per cent basic liquor was used, the Cr_2O_3 fixations with and without added formate were 6.16 and 5.23 per cent respectively, and 14.54 and 7.26 per cent in the case of a 44.0 per cent basic liquor. In other words, the presence of formate decreased chrome fixation in the case of the basic sulfate liquor and increased it when the basic chloride was employed.

Table 187

Sodium Acetate

No.	Molarity in Added Salt	pH of solutions: When prepared	pH of solutions: After 2 years	pH of solutions: Exhaust liquor	Precipitation (ml 0 1N NaOH)	% Cr_2O_3 fixed by h s	Acidity of Chrome-sulfate collagen compound (%)
1	0.00	2.62	3.03	3.12	2.6	11.71	57.1
2	0.01	3.33	3.06	3.13	2.6	11.49	53.6
3	0.04	4.10	3.18	3 23	2.5	10.81	53 3
4	0.10	4 37	3 50	3.71	5.6	9.21	45.1
5	0.20	4.52	3.93	4.04	. .	7.70	41.3
6	0.40	4.97	5 04	5.04	. .	2.18	32.7
7	1.00	5 60	5.77	5.68	.	0.54	0.0
8	2.00	6 07	6.24	6.14		0.34	0.0

Sodium Formate

No.	Molarity in Added Salt	pH of solutions: When prepared	pH of solutions: After 2 years	pH of solutions: Exhaust liquor	Precipitation (ml 0 1N NaOH)	% Cr_2O_3 fixed by h s	Acidity of Chrome-sulfate collagen compound (%)
1	0.00	2.98		3.06	2.8	12.25	55.0
2	0.10	3.37		3 40	3.0	10.90	44.0
3	0.20	3.69		3.74	4.5	10.43	31.9
4	0.50	4.39		4 43	. . .	8.97	11.8
5	1.00	4.92		4.93	. . .	6.38	10.5
6	1.50	5 25		5.22	.	4.78	6.6
7	2.00	5 50		5.43	.	3.43	1.8
8	3.00	5.87		5.80	. . .	1.94	0.0
9	4.00	6.18		6.09		1.09	0.0
10	6.00	6 68		6 46	. .	0.54	0.0

In 1934, Riess and Papayannis[70] made an extended study of the effects of adding various masking salts to chrome alum and to chrome chloride solutions. Their experimental methods were as follows. The chrome liquors were brought to the desired basicities by the addition of sodium carbonate; carbon dioxide was removed by boiling for five minutes and the liquors were allowed to cool. To the cooled solutions the masking salts were added, after which they were aged for 24 hours. To 5 grams of hydrated hide powder were added 50 ml of liquor containing 1.0 per cent Cr, and these mixtures were then continuously rotated for six hours at room temperature. The tanned powders were then sucked free of excess liquor and were washed four times with 100 ml of distilled water; in this way uncombined matter was removed, together with part of the fixed acid. The powders were then air-dried and analyzed for fixed chrome and fixed acid anions. Total acid was determined by the formaldehyde-ammonia method. Acid sulfate or chloride was also determined, and the masking radicals in the leather were obtained by difference The results may be summarized as follows.

The addition of sodium formate to either a 33.0 or a 50.0 per cent basic chrome alum liquor caused a progressive decrease of fixed Cr_2O_3 as a function of formate concentration, whereas the fixation of formate radical increased and fixation of acid sulfate decreased. The total basicity of the (washed) leathers in the case of both the 33.0 and the 50.00 per cent liquors—was only slightly changed by the presence of added formate. The amount of added sodium formate in both liquors ranged from 0.50 to 4.00 moles per mole of Cr.

Similar additions of sodium formate were made to both 33.3 and 50.0 per cent basic chrome chloride liquors. The analysis of powders tanned with these liquors showed, in the case of the 33.3 per cent basic, a decline in fixed chrome compared with the control, a decline in fixed acid chloride and a rise in fixed formate. In the case of the 50.0 per cent basic liquor, fixed chrome rose in the presence of one and two moles of formate and declined with four moles; fixed acid chloride declined and fixed formate rose.

When sodium oxalate was added to the chrome alum liquors described above, so that they contained from 0.25 to 2.00 moles per mole of Cr, analysis of the tanned powders indicated the following. In the case of the 33.0 per cent basic liquor, fixed chrome increased slightly at one mole oxalate and then declined rapidly, there being but slight chrome fixation at two moles. Fixed acid sulfate steadily declined and fixed oxalate steadily rose as a function of increasing oxalate additions. The latter observation held also for the 50.0 per cent basic liquor; but in this case fixed chrome declined rapidly with the addition of one mole and was negligible at both 1.5 and 2.0 moles oxalate. But when the two chrome chloride liquors described were treated with 0.25 and 0.50 mole sodium oxalate per mole of Cr, chrome fixations rose sharply

with both oxalate additions, as did the fixed oxalate; the fixed chloride declined.

When from 0.25 to 1.00 moles sodium sulfite was added to the 33.0 basic chrome alum and 0.25 mole was added to the 50.0 per cent, fixed chrome was increased throughout.

Riess and Papayannis did not report any pH values for the various liquors described above, either before or after tanning. They found only negligible changes in the shrinkage temperatures of skin fibers tanned in the various liquors to which formate or sulfite were added. There was, however, considerable decrease of shrinkage temperature in the case of chrome alum liquors containing large additions of oxalate; but the opposite was true in the case of the chrome chlorides.

It is very difficult to attempt to discuss profitably or interpret the experiments just described, for two reasons. In the first place, none of the various tannages were run to anywhere near tanning equilibrium. This is at once apparent from the fact that the control experiment of the 50.0 per cent basic chrome alum liquor fixed *only* 7.53 per cent Cr_2O_3. Had the tanning time been extended to equilibrium (instead of the short tanning period of six hours) very much greater fixations would have occurred - and masked liquors require even longer tanning time than unmasked. The second reason for objection is the fact that the tanned powders were given a drastic water washing before drying and analysis. While such washing may have removed uncombined matter, it automatically removed a large amount of fixed anions also. We have no means of knowing the extent of this removal, nor whether the hydrolysis constants of the various fixed anions were similar or different.

In a series of papers published in 1939 and 1940, Holland[33] has investigated the effects of adding numerous organic compounds to basic chrome liquors. He first added such compounds as quinone, hydroquinone, quinhydrone and sucrose to chrome alum solutions and found that they had little or no effect upon either the pH value or the sulfate distribution of the chrome solution. Because of these findings, he suggests that the slightly lowered chrome fixation which Thomas and Foster found when their 33.0 per cent basic chrome sulfate liquor was made 4.0 molar with sucrose was due to hydration of the sucrose. (Thomas and Foster had suggested that the phenomenon was probably a function of a change in the composition of the chrome complex, and was not due to sucrose hydration.) Holland then suggested that if any reaction actually occurs between chromium complexes and the compounds studied, it must take place very slowly in dilute solutions and would be quite different from the rapid effect of adding ionizing organic salts to chrome solutions. If this is true, vegetable tannins should not appreciably affect the composition of chrome compounds.

Holland then studied the effect of adding various sodium salts to a 33.0

per cent basic chrome sulfate liquor derived from the reduction of sodium bichromate with sulfur dioxide gas. (When the completed liquor was diluted to 20.0 grams Cr_2O_3 per liter it showed cathodic migration only.) The concentrated liquor was allowed to age for 48 hours and was then diluted to 20.0 g.p.l. with a solution containing the proper amount of organic salt. Five grams of hide powder were then given 200 ml of the diluted chrome liquor and the tannage was performed at 18° for 24 hours, with agitation during the first seven hours and the last hour of tannage. At the completion of tannage the liquor was removed from the powder, which was then washed with five 100-ml portions of distilled water, after which the tanned powders were dried and analyzed. The tanning treatment described was, of course, insufficient to insure complete or equilibrium tannage. This is at once apparent from the fact that the controls show only some 8.7 per cent fixed Cr_2O_3; moreover, the water-washing of the tanned powders hydrolyzed unknown amounts of fixed acid radicals. But with these limitations in mind it is interesting to note the experimental results, as shown in Table 188, in which the data shown refer to cold solutions to which the organic salts were added.

Table 188

Moles salt per mole $Cr(OH)SO_4$	Precipitation (ml alkali)	Initial pH value	Exhaust pH value	Combined SO_4 on hide sub (%)	Combined Cr_2O_3 on hide sub (%)
0.00 Formate	6.3	2.84	2 95	.	8.75
0.25 "	6.4	3.60	.		9.17
0 50 "	6.6	3 86	3 15		9.16
1.00 "	6.9	3 88	3 46		9 68
2 00 "	>20.0	4.20	3.95	.	9.39
4.00 "	.	4.58	4.57		7.85
0.00 Acetate	6.0	2 88	2.94	5 07	8 52
0 50 "	6.0	4 61	3.24	4.89	10 25
1.00 "	6.0	4 87	3 60	3 95	9.97
2 00 "	6.0	5 25	4.41	2.34	9.39
4 00 "	6 3	5 55	5.07	0.98	6.04
0.00 Oxalate	6.1	2.76	2.90	. .	8.73
0 25 "	6 1	3.72	3.16	.	9 52
0.50 "	6.2	3.98	3.47		8 47
1 00 "	6.2	5.27	4 06	.	7.81
0.00 Succinate	6.0	2.83	2 89		8.89
0.25 "	6.1	4 56	3.23		14 15
0.50 "	6.1	4.74	3.38		17 48
0.00 Phthalate	. . .	2 78	2.88	5 14	8.35
0.25 "		4 28	2.92	4.14	13.03
0.50 "		4.50	3 09	3.08	17.35

Table 189 shows the effect of adding 0.50 mole per mole of $Cr(OH)SO_4$ of various sodium salts of different acids.

Tables 188 and 189 indicate that additions of formate do not greatly change chrome fixation until 4.0 moles are present; acetate increases fixation

Table 189

Acid	Precipitation (ml. alkali)	Initial pH value	Exhaust pH value	Combined Cr_2O_3 on hide sub (%)
Control	6.3	2 84	2.89	8.70
Malonic	6.4	4.11	3.32	9.09
Succinic	5.6	4.77	3.38	16.10
Maleic	6.0	4.58	3.13	12.20
Adipic	6.2	4.62	3.22	17.70
Phthalic	7.4	4.00	3.22	16.80
Oxalic	6.2	3.98	3.47	8.47
Formic	6.6	3.86	3.15	9.16
Acetic	6.0	4.82	3.24	10.25

up to and including 2.0 moles, decreasing fixation occurring with 4.0 moles; 0.25 mole of oxalate increases fixation, which is decreased by greater concentrations; succinate greatly increases fixation, as does phthalate. As the concentration of either acetate or phthalate is increased, the amount of fixed acid sulfate in the leather decreases; and the same finding would probably have held true for the other added salts if leather sulfate had been determined. There seems to be no relation in the tables between precipitation and chrome fixation, nor is the latter directly related to the pH value of the exhaust chrome liquors.

Table 190. Effect of Formate Additions.

Immediate Tannage

No.	Moles Added Salt	pH	% Cr_2O_3	% SO_3	% Formate	% Cr	Milli-moles Cr	Milli-moles SO_3	Milli-moles Formate	% Formate in Terms of % SO_3	Total Anion in % SO_3	% Basicity
1	..	3 02	7 72	7.75	.	5 28	101 4	96 8	.		7 75	36 5
2	1/16	3 04	7 82	7.66	0.01	5 35	102 8	95 8		.	7 66	38 0
3	1/8	3 13	8 00	7.40	0 01	5 47	105 2	92 5		.	7 40	41 4
4	1/4	3 22	7 63	7.17	0 01	5 22	101.2	89 7			7.17	40 5
5	3/8	3 30	7 95	7.01	0 48	5 44	104 5	87 6	10 7	0 84	7 85	37 2
6	1/2	3 37	7 95	6 70	0 81	5 44	104 5	83 8	18.0	1 45	8 15	35 2
7	3/4	3 50	8 02	6 21	0 96	5 48	105 4	77 6	21 3	1 71	7 92	37 5
8	1	3.63	7 97	5 76	1 78	5 45	104.8	72 0	39 6	3 17	8 93	29 2
9	1½	3 87	7.97	4 87	2 62	5 45	104 8	60 8	58 3	4 67	9 54	24 3
10	2	4 08	7.97	3 96	3 18	5 45	104 8	49 5	70.6	5 66	9 62	23.5

Table 191. Effect of Formate Additions.

Aged Liquors

No.	Moles Added Salt	pH	% Cr_2O_3	% SO_3	% Formate	% Cr	Milli-moles Cr	Milli-moles SO_3	Milli-moles Formate	% Formate in Terms of % SO_3	Total Anion in % SO_3	% Basicity
1	.	3 10	7.63	7 30	.	5 22	100 2	91 3	.	...	7 30	39 4
2	1/16	3 14	7.63	7.11	0 07	5 22	100 2	89 0	1 55	0 12	7 23	40 0
3	1/8	3 18	7 60	7 10	0.14	5 19	99 9	88 8	3.11	0 25	7 35	38 8
4	1/4	3 27	7 32	6 81	0 32	5.00	96 2	85 1	7 11	0 53	7 34	36 5
5	3/8	3.32	7 35	6.61	0 41	5 03	96 7	82 7	9 12	0 73	7 34	36 8
6	1/2	3.39	7 35	6 42	0 85	5.03	96.7	80.2	18.9	1 51	7 93	31 6
7	3/4	3 51	7 33	6.02	1 09	5 01	96.4	75 3	24 9	1 94	7 96	31 2
8	1	3.63	7 02	5.35	1 33	4 80	92 3	66 8	29 6	2 36	7.71	30 4
9	1½	3.83	7 10	4 63	1 52	4 85	93 3	57 8	33 8	2 70	7 33	34 6

Starting in 1940, Theis and his collaborators[92] published a series of investigations of the effect of adding masking salts to basic sulfate liquors. They tanned 5.0-gram portions of standard hide powder in 100 ml of 33.0 per cent basic sulfur dioxide-reduced liquor in which was incorporated the required amount of the various masking salts. Tannage was for 48 hours at 25° with constant agitation. The tanned powders were placed in a Büchner funnel

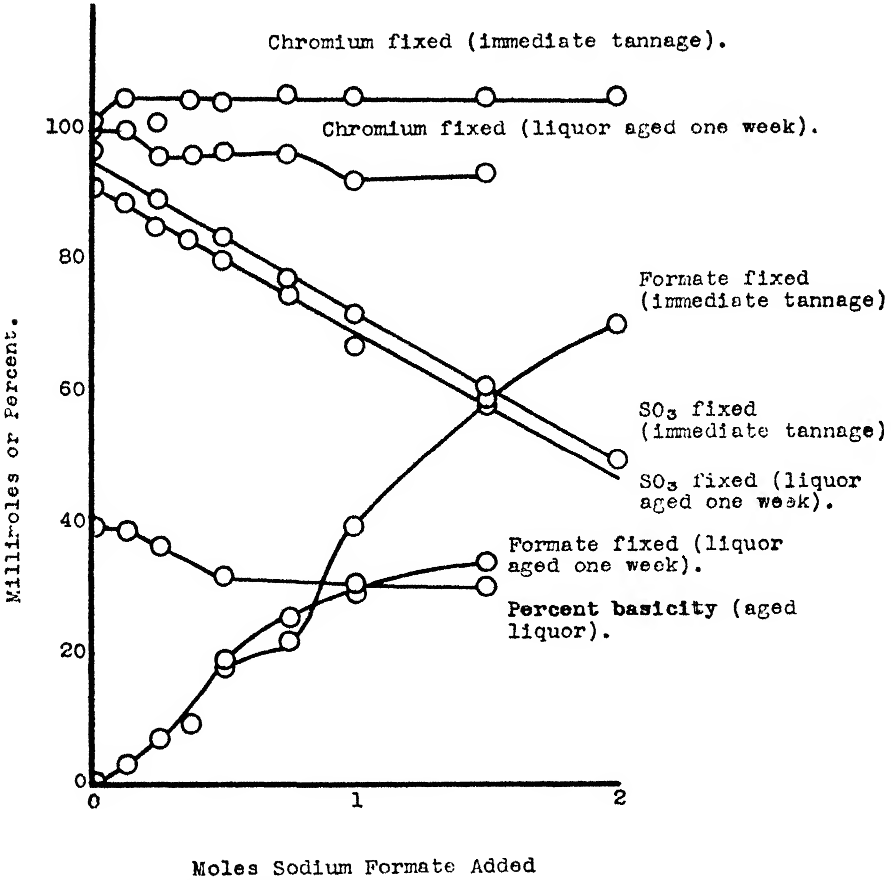


Figure 150. The effect of sodium formate additions upon chromium, sulfate, and formate fixation by collagen.

and were washed free of uncombined matter (and also part of the combined acid radicals) by treatment with 5 liters of distilled water at 75° over a period of two hours. The powders thus washed were then air-dried and analyzed for fixed Cr_2O_3 and the various combined acid groups. The latter values were determined by digesting one gram of tanned powder with 200 ml of 2

per cent ammonia solution for two hours at 70°, after which the powder was thoroughly washed with distilled water. The combined filtrate and washings were then neutralized with hydrochloric acid and the various anions present were determined.

The effects of adding sodium formate in concentrations ranging from zero to two moles of formate per gram atom of chromium are shown in Tables 190 and 191, where the various fixations shown are based upon hide substance. Table 190 illustrates tanning results when the chrome liquors were used immediately after they were prepared; Table 191, on the other hand, shows the effect of allowing the liquors to age for one week after the sodium formate additions. The two tables are illustrated in Figure 150, from which we note

Table 192. Effect of pH of Tanning.
0.25 Mol Sodium Formate Added

Moles of Added Salt	pH	% Cr_2O_3	% Cr	% SO_4	% $HCOO^-$	Milli-moles Cr	Milli-moles SO_4	Milli-moles $HCOO^-$	% $HCOO^-$ in Terms of % SO_4	Total Anion as % SO_4	% Basicity
¼	3.00	7.19	4.92	6.65	0.19	94.5	83.2	4.22	0.34	6.99	38.3
¼	3.50	7.75	5.30	6.80	0.24	101.8	86.3	5.33	0.43	7.23	40.9
¼	4.00	8.39	5.74	6.82	0.23	110.4	86.5	5.12	0.41	7.23	45.4
¼	4.50	9.67	6.62	7.03	0.42	127.2	88.0	9.34	0.75	7.78	49.0
¼	5.00	11.10	7.59	7.02	0.43	146.1	87.8	9.56	0.76	7.78	55.6

Table 193. Effect of pH of Tanning.
0.75 Mol Sodium Formate Added

Moles of Added Salt	pH	% Cr_2O_3	% SO_4	% $HCOO^-$	% Cr	Milli-moles Cr	Milli-moles SO_4	Milli-moles $HCOO^-$	% $HCOO^-$ in Terms of % SO_4	Total Anion as % SO_4	% Basicity
¾	3.0	7.06	6.15	0.76	4.83	93.0	77.0	16.9	1.35	7.50	32.7
¾	3.5	7.61	6.14	0.76	5.20	100.0	76.9	16.9	1.35	7.49	37.7
¾	4.0	8.56	6.05	1.18	5.85	112.5	75.7	26.2	2.10	8.15	39.7
¾	4.5	9.11	6.00	1.47	6.23	120.0	75.1	32.6	2.62	8.62	40.2
¾	5.0	10.10	5.94	1.56	6.92	133.2	74.3	34.7	2.78	8.72	45.3
¾	5.5	11.45	5.89	1.72	7.84	150.1	73.7	38.2	3.06	8.95	50.5
¾	6.0	12.95	5.68	2.08	8.86	170.1	71.2	46.3	3.70	9.38	54.2

the following. (1) All formate additions caused a slight increase in the amount of fixed Cr_2O_3 from the unaged liquor, but slightly lowered fixation in the aged. (2) In the case of both liquors the fixed acid sulfate decreased and the fixed formate increased with increasing formate additions. (3) The total or overall basicity of the leather increased up to 0.25 per cent mole of added sodium formate and then decreased sharply from 1.00 mole onward,

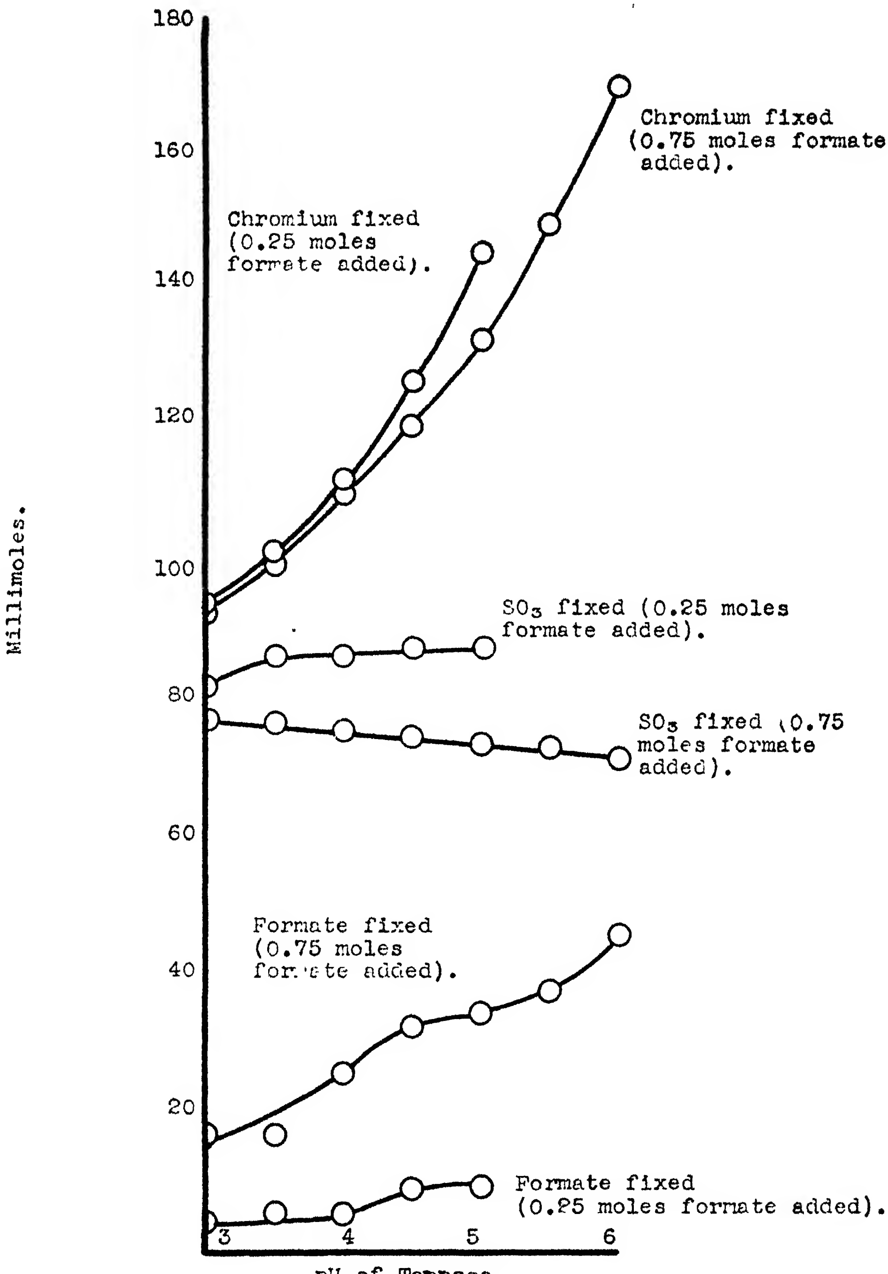


Figure 151. The effect of tanning pH upon chromium, sulfate, and formate fixation.

in the case of the unaged liquors; leather basicity changes were less apparent with the aged liquors. (4) There was a steady rise in the pH values of the exhaust liquors as a function of increasing formate additions.

The effect of pH value of liquors masked with sodium formate are shown in Tables 192 and 193. Table 192 illustrates the behavior of unaged liquor to which a constant amount of sodium formate (0.25 mole formate per gram

Table 194. Effect of Sodium Oxalate Additions.

Immediate Tannage

Moles NaOx per gm atom	Final pH	% Cr_2O_3	% SO_3	% C_2O_4	C_2O_4 as SO_3	Total Anions as SO_3	Basicity	% Cr	Millimoles Cr	Millimoles SO_3	Millimoles C_2O_4
0	2.98	7.40	6.89	0.00	0.00	6 89	41.0	5 07	97 5	86 1	0.0
1/16	3 07	7.42	6.53	0.35	0.32	6 85	41.4	5.08	97.8	81 6	4.0
1/8	3.18	7.48	6.25	0.82	0 75	7.00	40.8	5.12	98.5	78 1	9.3
1/4	3.36	7.50	5.46	1.66	1.51	6.97	41.1	5 13	98.7	68.3	18 9
3/8	3.45	7.56	4.71	2.43	2.21	6 92	42.0	5.17	99.5	58.9	27.7
1/2	3.60	7.57	4.18	3 15	2.86	7 04	41 1	5.18	99.7	52 2	35.8
3/4	4 01	7.54	2.89	4.38	3.98	6.87	42.3	5 16	99.3	36 1	49 8
1	4.35	7.40	1.67	5 56	5.06	6 73	42 5	5.07	97 6	20.9	63 1
1½	5.25	4.83	0.04	5.04	4.58	4.62	39.4	3 30	63 5	0 5	57.4
2	6.15	1.74	0.03	2.47	2 24	2 27	17.5	1 19	22 9	0.4	28.1

Table 195. Effect of Sodium Oxalate Additions.

Aged Liquors

Moles NaOx per gm atom	Final pH	% Cr_2O_3	% SO_3	% C_2O_4	C_2O_4 as % SO_3	Total Anions as % SO_3	Basicity	% Cr	Millimoles Cr	Millimoles SO_3	Millimoles C_2O_4
0	3.00	7.56	6.99	0.00	0.00	6 99	41.5	5 17	99.5	87.4	0.00
1/16	3.14	7.66	6 84	0 13	0.12	6 96	42.5	5 24	100 8	85.5	1 48
1/8	3.18	7 67	6.65	0.41	0.37	7.02	42.0	5.25	101.0	83.2	4.66
1/4	3.36	7.89	6 00	0 98	0 89	6 89	44 6	5 40	103 9	75.0	11 1
3/8	3 45	8.19	5 91	1.30	1 17	7.08	45.0	5 60	107.7	73.9	14.8
1/2	3.60	8.20	5.05	2.10	1.91	6.96	46.2	5.61	108.0	63.2	23.9
3/4	4.02	8.30	3.73	3.55	3.23	6.96	46.9	5 68	109 3	46.7	40.3
1	4.35	8.35	2.25	4 87	4.43	6.68	49.3	5.72	110.0	28 2	55 8
1½	5.24	6.40	0.10	5 31	4.83	4.93	51.2	4.38	84.1	1.2	60.3
2	6.30	1.47	0.07	2.07	1.88	1 95	16.0	1.01	19.4	0.9	23.5

atom of chromium) was added but whose pH values were varied as shown; while Table 193 illustrates the same phenomena in the presence of 0.75 mole added formate. These two tables are illustrated in Figure 151, from which we note: (1) Fixation of Cr_2O_3 increases in all cases with rising pH values. (2) Fixed acid sulfate increases with rising pH values in the presence of 0.25 mole formate, but slightly decreases with 0.75 mole. Fixed formate increases only slightly at 0.25 mole but rises rapidly at 0.75.

Similar studies were made as to the effect of adding increasing amounts of sodium oxalate. The results are given in Tables 194 and 195, and are illustrated in Figures 152 and 153, from which we note: (1) Oxalate additions

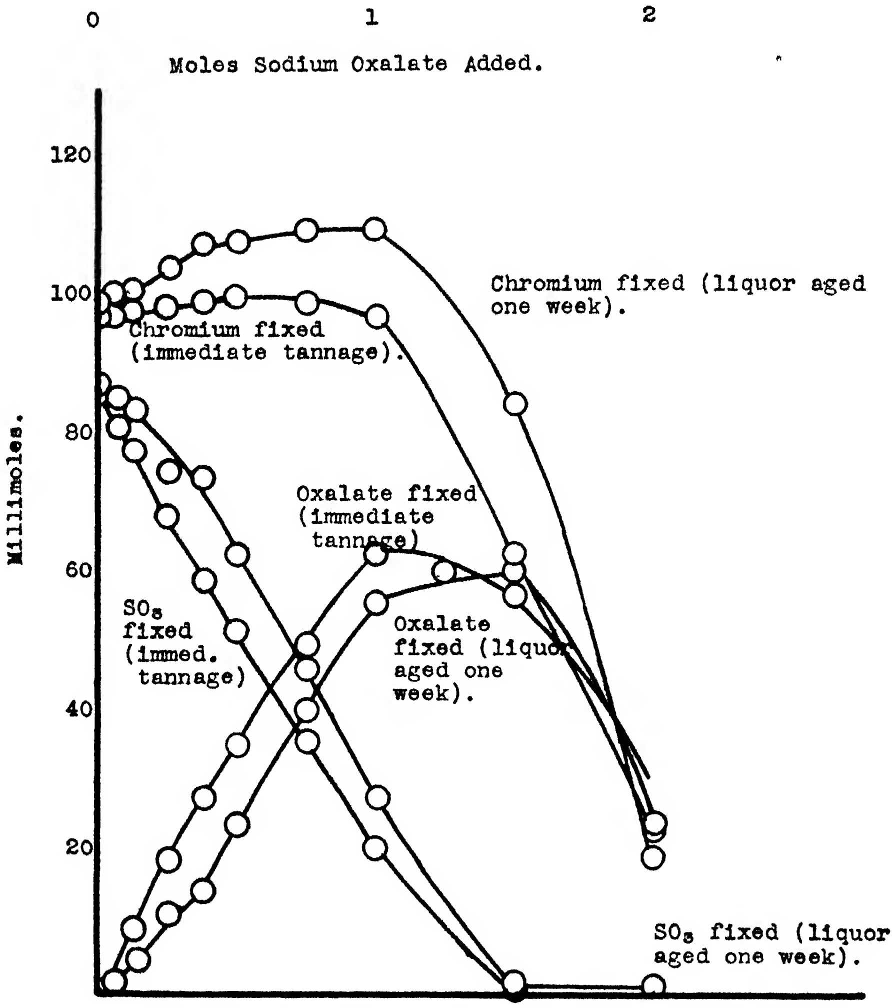


Figure 152. The effect of sodium oxalate additions upon chromium, oxalate, and sulfate fixation.

have but little effect upon chrome fixation of unaged liquors until 1.00 mole oxalate has been exceeded, whereas fixation from the aged liquor increases up to 1.00 mole. (2) Fixed acid sulfate decreases (reaching practically zero value at 2.00 moles) and fixed oxalate rises and then declines at the higher

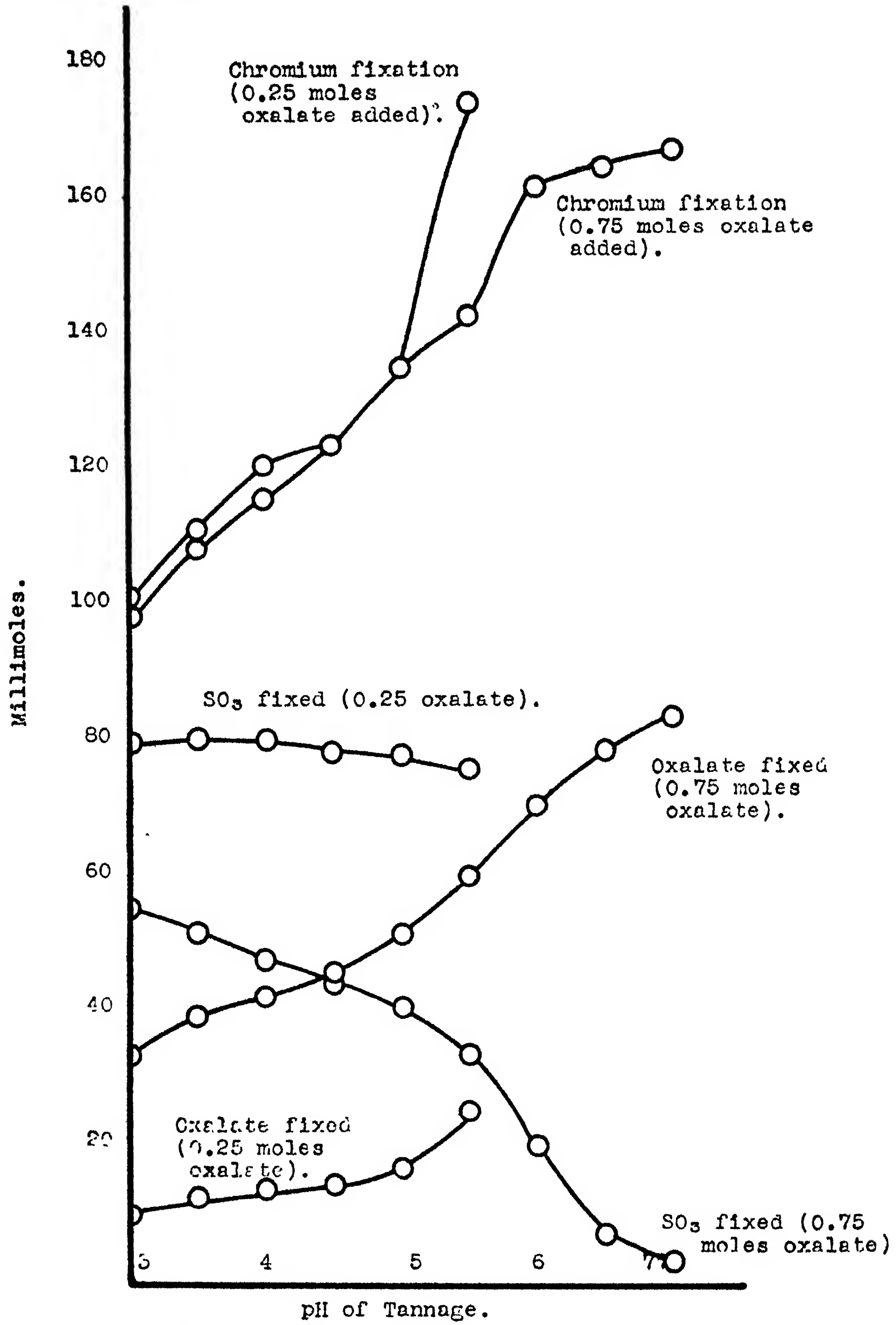


Figure 153. The effect of neutralization upon oxalato chrome liquors.

concentrations of added oxalate. When the amount of added oxalate was held constant (0.25 and 0.75 mole) and the pH values of the liquors were adjusted to range from 3.00 to 7.00, the shapes of the curves (Figure 153) are quite similar to those shown for the formate series (Figure 151), with the exception of the very much lowered fixation of acid sulfate with rising pH values of the oxalate tannage. But it must be borne in mind in the latter connection that these contrasting effects may not strictly hold, since we are comparing molarities of a mono- and a dibasic salt.

Table 196. Effect of Tanning pH.

Moles of Added Salt	pH	% Cr_2O_3	% Cr	% SO_3	% Oxalate	Milli-moles Cr	Milli-moles SO_3	Milli-moles Oxalate	% Oxalate as % SO_3	Total Anion as % SO_3	% Basicity
$\frac{1}{4}$	3.00	7.50	5.13	6.43	0.94	98.7	80.4	10.7	0.85	7.28	38.5
$\frac{1}{4}$	3.50	8.29	5.67	6.48	1.13	109.1	81.2	12.8	1.03	7.51	42.6
$\frac{1}{4}$	4.00	8.86	6.06	6.46	1.23	116.5	80.7	14.0	1.12	7.58	45.8
$\frac{1}{4}$	4.50	9.46	6.47	6.31	1.31	124.3	78.8	14.9	1.19	7.50	49.8
$\frac{1}{4}$	5.00	10.40	7.13	6.26	1.55	137.0	78.4	17.6	1.41	7.67	53.3
$\frac{1}{4}$	5.50	13.30	9.12	6.10	2.16	175.2	76.3	24.6	1.97	8.07	61.6

Table 197 Effect of Tanning pH.

Moles of Added Salt	pH	% Cr_2O_3	% SO_3	% $C_2O_4^=$	% Cr	Milli-moles Cr	Milli-moles SO_4	Milli-moles $C_2O_4^=$	% C_2O_4 in Terms of % SO_3	Total Anion as % SO_3	% Basicity
$\frac{3}{4}$	3.0	7.70	4.44	2.97	5.27	101.4	55.5	33.8	2.70	7.14	41.4
$\frac{3}{4}$	3.5	8.48	4.13	3.48	5.80	111.5	51.7	39.6	3.16	7.29	45.6
$\frac{3}{4}$	4.0	9.16	3.84	3.73	6.27	120.7	48.0	42.4	3.39	7.23	50.0
$\frac{3}{4}$	4.5	9.42	3.56	4.04	6.44	123.8	44.5	45.8	3.68	7.24	51.3
$\frac{3}{4}$	5.0	10.30	3.28	4.60	7.05	135.7	41.0	51.7	4.18	7.46	54.1
$\frac{3}{4}$	5.5	10.90	2.73	5.33	7.45	143.3	34.2	60.6	4.85	7.58	55.9
$\frac{3}{4}$	6.0	12.30	1.62	6.22	8.42	162.0	20.3	70.7	5.65	7.27	62.6
$\frac{3}{4}$	6.5	12.55	0.62	6.93	8.58	165.0	7.75	78.8	6.30	6.92	65.1
$\frac{3}{4}$	7.0	11.70	0.27	7.40	8.68	167.0	3.38	84.1	6.73	7.00	65.1

When sodium acetate was employed the results shown in Tables 198 and 199 were obtained and they are illustrated in Figure 154. The figure shows that whereas the addition of acetate does not greatly affect the chrome fixation from unaged liquor, it drastically lowers fixation when the liquor containing acetate is aged for one week before use. This is an interesting example of the fact that time is required for the penetration of an ion into the chrome complex. Fixed acid sulfate declines rapidly as a function of acetate concentration and fixed acetate rises.

The effect of adding sodium lactate, without any adjustment of the pH value of the various liquors, is illustrated in Table 200, where bated skin

strips were employed instead of hide powder. The liquors shown in thi[s] table were aged for one week after the lactate additions. Table 200 shows [a] progressive increase in the pH value of the liquors, both before and afte[r] tannage, as a function of increasing lactate. The shrinkage temperature of th[e] tanned strips drops from 120° for the control to 108° in the presence of 1.0[0]

Table 198. Effect of Sodium Acetate.

Immediate Tannage

Moles of Added Salt per gm Atom of Cr	pH After Tanning	% Cr_2O_3	% Cr	Millimoles of Cr	% Sulfate as SO_3	% Acetate	Millimoles Sulfate as SO_3	Millimoles Acetate	% Acetate in Terms of % SO_3	Total % of Anions of % SO_3	% Basicity
..	3.03	7.70	5.27	101.3	7.40	. .	92.5	. .	.	7.40	39.2
1/16	3.04	7.75	5.30	101.8	7.32	0.20	91.5	3.4	0.27	7.59	38.0
1/8	3.10	7.80	5.34	102.7	7.21	0.22	90.3	3.7	0.30	7.50	39.1
1/4	3.16	7.83	5.36	103.0	6.93	0.74	86.7	12.4	1.00	7.93	35.8
3/8	3.27	7.98	5.46	105.1	6.75	1.13	84.5	19.2	1.53	8.28	34.7
1/2	3.38	7.78	5.32	102.2	6.50	0.92	81.3	15.6	1.24	7.74	37.1
3/4	3.57	7.82	5.35	102.8	6.42	2.40	80.3	40.7	3.26	9.68	21.5
1	3.80	7.70	5.27	101.3	5.18	2.87	64.8	48.6	3.89	9.07	25.4
1½	4.22	7.78	5.32	102.2	3.77	3.84	47.2	65.0	5.20	8.97	27.0
2	4.54	7.50	5.13	98.8	2.78	5.13	34.9	87.0	6.95	9.73	17.8

Table 199. Effect of Sodium Acetate

Aged Liquors

Moles of Added Salt	pH After Tanning	% Cr_2O_3	% Cr	% SO_3	% Acetate	Millimoles Cr	Millimoles SO_3	Millimoles Acetate	% Acetate in Terms of % SO_3	Total Anions as % SO_3	% Basicity
..	3.12	7.82	5.35	7.58		102.8	94.6			7.58	38.6
1/16	3.08	7.77	5.32	7.50	1.55	102.2	93.8	26.2	2.10	9.60	21.7
1/8	3.10	7.63	5.22	7.24	1.70	101.3	90.5	28.8	2.30	9.54	21.0
1/4	3.21	7.43	5.08	6.91	1.86	97.7	86.4	31.5	2.52	9.43	19.7
3/8	3.30	7.22	4.94	6.57	2.21	95.0	82.2	37.4	3.00	9.57	16.0
1/2	3.38	7.20	4.92	6.30	2.35	94.5	78.8	39.9	3.18	9.48	16.5
3/4	3.56	6.93	4.74	5.70	2.88	91.1	71.3	48.8	3.91	9.61	12.2
1	3.75	6.86	4.70	5.00	3.36	90.3	62.5	57.0	4.56	9.56	11.8
1½	4.13	6.03	4.12	3.59	5.08	79.2	44.8	73.7	6.90	10.49	−10.2
2	4.52	4.78	3.27	2.16	7.06	62.9	27.0	119.5	9.58	11.74	−55.5

mole of lactate and is only 75° with 6.00 moles. Fixed chrome decreases steadily with increasing lactate concentrations, as does the fixed acid sulfate. Fixed lactate ion increases up to 2.00 moles added lactate and then sharply decreases with further lactate concentration. The same general results are obtained when the pH value of the various aged liquors were adjusted to a common value of 3.0; this value was maintained throughout the entire tanning period. The results are shown in Table 201 and Figure 155. When the liquor contained a constant amount of added lactate (1.00 mole per gram atom of

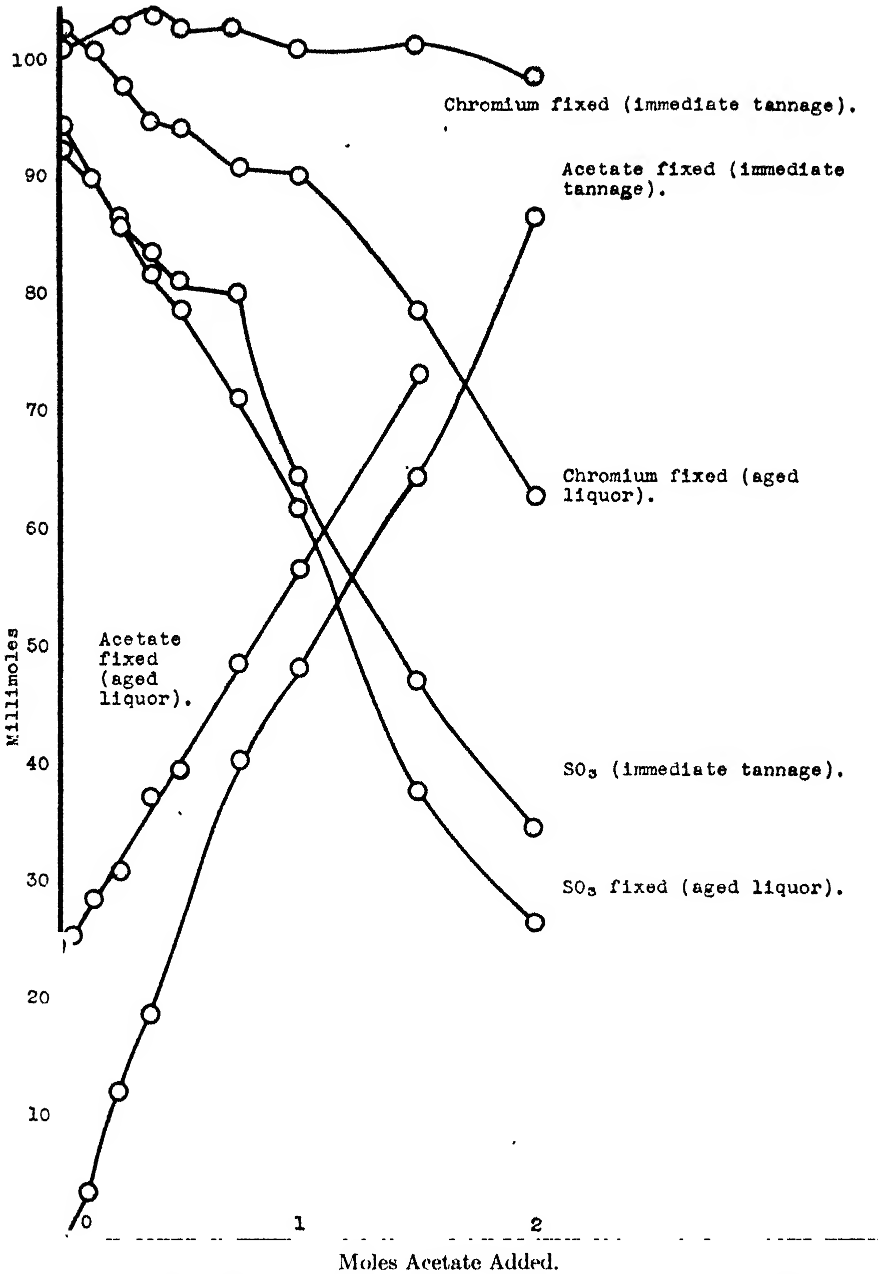


Figure 154. The effect of sodium acetate additions upon chromium, sulfate, and acetate fixation.

Table 200. Effect of Sodium Lactate.

(Natural pH of prepared liquors)

Moles Sodium Lactate per g. atom Cr	pH before Tannage	pH after Tannage	Average Shrinkage Temp. (° C)	% SO_3	% Cr_2O_3	% Lactate Ion	% Lactate Ion as SO_3	Total Anion as SO_3%	% Basicity	Millimoles SO_3/100 g.	Millimoles Lactate Ion per 100 g.	% Cr	Millimoles Cr/100 g.
0	2.85	3.20	120	6 64	7 32	...	...	6.64	41.9	83 0	...	4.95	95.2
1/16	3.23	3 28	115	6.29	7 05	0.42	0.19	6.48	41.7	78.5	4 7	4.82	92 7
1/8	3 50	3 30	110	6.16	7.05	0.68	0.31	6.47	41.8	77.0	7.7	4.82	92.7
1/4	3.72	3.38	116	5.82	7.01	1.23	0 55	6.37	42.4	72 8	13.8	4 80	92.3
1/2	4 05	3.55	114	4.90	6.34	1.98	0.89	5.79	42.2	61 3	22.2	4 34	83.4
3/4	4 10	3.70	112	3.97	6.31	3 21	1.44	5.41	45 6	49 7	36 1	4.32	83.0
1.0	4 15	3 80	108	3.26	5.73	3.51	1 58	3.84	57.6	40.7	39 4	3.92	75 4
1 5	4 20	3.90	104	1 94	5.36	4.60	2 07	4.01	52 6	24 2	49.4	3 67	70 5
2 0	4.30	4 15	98	1 28	4.70	5.05	2.28	3.56	52.0	16 0	56.8	3 22	61 9
3 0	4.51	4.45	91	0 66	2.81	3 86	1.74	2.40	49.9	8 3	43.4	1.92	36 9
4 0	4 70	4 65	86	0.29	2.50	3.26	1.47	1 76	55.4	3.7	36 6	1.71	32 9
5.0	4 80	4 80	81	0 29	1.44	3.07	1 38	1.67	26 5	3.6	34.5	0 99	19 1
6.0	4.92	4.90	75	0 24	1 38	2 48	1.12	1.36	37.6	3.1	27 9	0.95	18 3

Table 201. Effect of Sodium Lactate.

(pH of liquors adjusted to 3)

Moles Sodium Lactate per g. atom Cr	Average Shrinkage Temp. (° C)	% SO_3	% Cr_2O_3	% Lactate Ion	% Lactate Ion as SO_3	Total Anions as SO_3%	% Basicity	Millimoles SO_3/100 g	Millimoles Lactate ion per 100 g.	% Cr	Millimoles Cr/100 g
0	114	6.48	6.57	...	...	6.48	37.5	81.0	...	4.50	86.5
1/16	114	6.40	6.50	0.63	0.28	6.68	34.9	80.0	7.1	4.45	85.5
1/8	112	6.20	6.39	0.83	0.37	6.57	34.8	77.5	9.3	4.37	84.1
1/4	112	5.86	6.12	1.09	0.49	6.35	34.3	73.4	12.3	4.19	80.6
1/2	105	4.84	5.19	1.48	0.67	5.51	32.8	60.5	16.6	3.55	68.3
3/4	101	4.60	4.65	2.08	0.94	5.54	24.5	57.5	23.4	3.18	61.1
1	97	3.74	4.05	2.65	1.19	4.93	22.9	47.0	29.8	2.77	53.2
1.5	88	3.36	3.11	2.82	1.27	4.63	5.6	42.0	31.7	2.13	41.0
2.0	81	2.69	2.67	2.58	1.16	3.85	8.6	33.6	29.0	1.83	35.2
3.0	73	2.50	1.99	2.38	1.07	3.57	−13.8	31.2	26.8	1.36	26.2
4.0	66	2.36	1.46	2.38	1.07	3.43	−48.9	29.5	26.8	1.00	19.2
5.0	62	2.25	1.18	1.78	0.80	3.05	−63.8	28.1	20.0	0.81	15.6
6.0	58	2.12	0.93	1.39	0.63	2.74	−86.8	26.5	15.6	0.64	12.3

Table 202. Effect of Sodium Lactate.

(pH of liquors adjusted from 2.5 to 5)

pH	% Cr_2O_3	% Cr	% SO_3	% Lactate Ion	% Lactate Ion as SO_3	% Total Anions as SO_3	% Basicity	Millimoles SO_3 per 100 g	Millimoles Lact Ion per 100 g	Millimoles Cr/100 g
2.5	2.83	1.94	3.74	2.38	1.07	4.81	−7.8	46.7	26.8	37.3
3.0	4.40	3.01	3.81	3.07	1.38	5.19	25.2	47.6	34.5	57.9
3.5	5.54	3.79	3.67	3.46	1.56	5.23	40.2	45.9	38.9	72.8
4.0	6.64	4.54	2.99	4.50	2.02	5.01	52.2	37.4	50.5	87.2
4.5	7.39	5.05	2.68	4.95	2.23	4.91	57.9	33.4	55.6	97.1
5.0	8.44	5.77	1.87	5.92	2.66	4.53	66.0	23.4	66.5	111.0

Original pH of liquor = 3.80

chromium) and the maintained pH values were varied, the results shown in Table 202 and Figure 156 were secured. The chrome fixation rises steadily as a function of increasing pH values, the fixed acid sulfate declines and the fixed lactate increases.

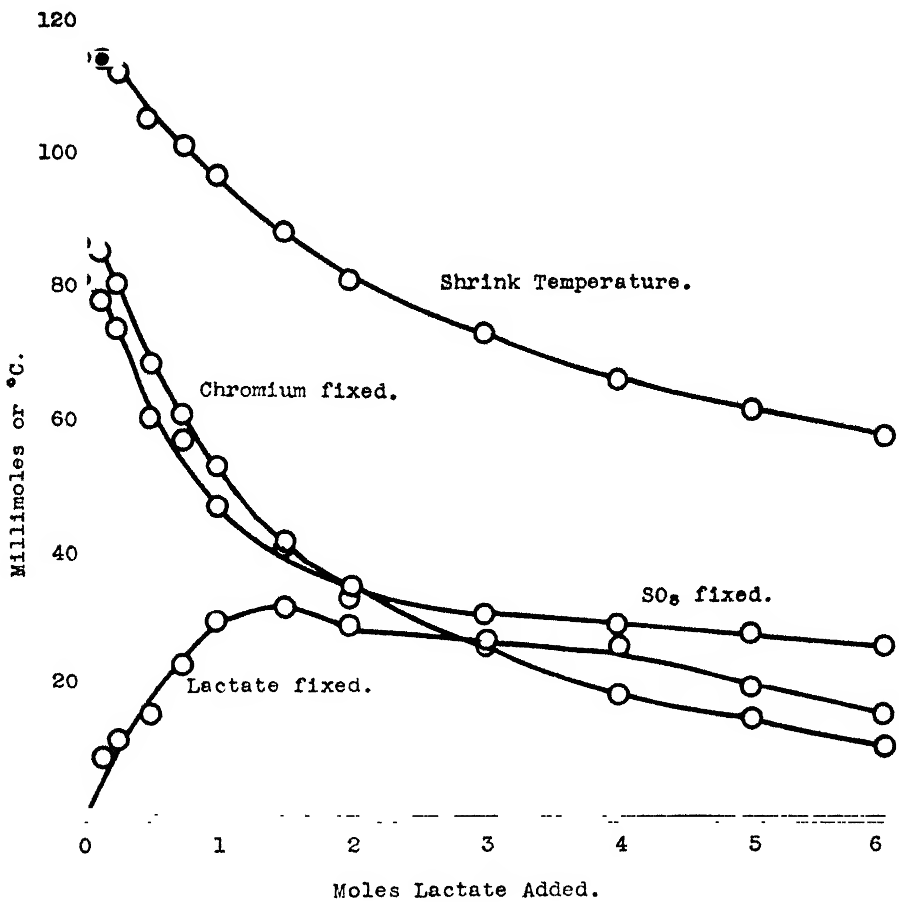


Figure 155. The effect of sodium lactate additions upon the shrinkage temperature and upon the chromium, sulfate, and lactate fixations when the chrome liquors are adjusted to pH = 3.

In considering the experiments of Theis and his collaborators which have just been described, the following reservations must be recognized. The various tannages probably did not reach equilibrium; this is evident from the fact that the hide powder controls fixed only about 7.70 per cent Cr_2O_3.

The water-washing given after tannage removed unknown amounts of fixed acid radicals (see Table 175). The method employed for determining the fixed acid radicals remaining in the washed leathers has not been shown to yield highly accurate results; masking ions have great power of complex

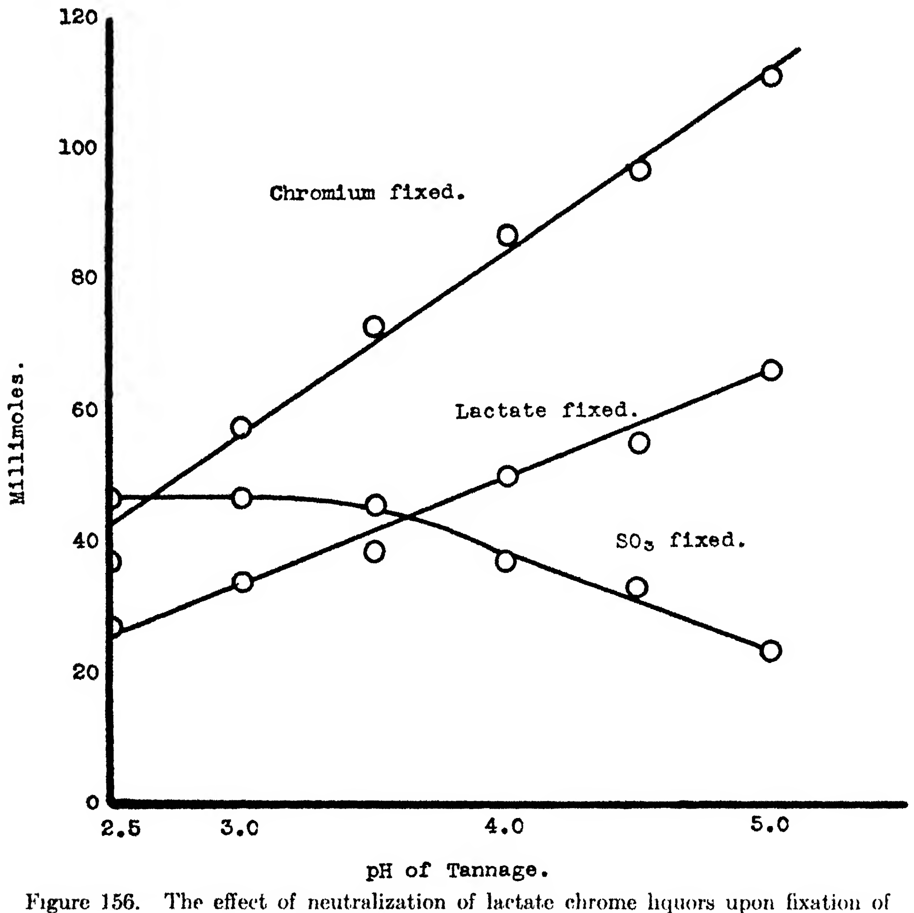


Figure 156. The effect of neutralization of lactate chrome liquors upon fixation of chromium, lactate, and sulfate.

penetration and it is by no means certain that they are completely removed by digestion with dilute ammonia solution. But with these several limitations in mind, the results given are of interest and illustrate the powerful effect of masking salts upon the tanning behavior of basic chrome sulfate.

It is to be hoped that further work with masked chrome liquors will be pursued. But we would suggest that care be taken to insure complete tannage and that uncombined matter be removed by hydraulic pressing rather

than by the unsatisfactory washing procedure. And an accurate method for determining the various anions fixed by hide substance from masked liquors is urgently needed.

Chromiates

When a masking salt, such as sodium oxalate, is added to a basic chrome sulfate or chloride, a system of great complexity results, as we have seen in the previous section. But if sodium dichromate is reduced with oxalic acid a less complicated system is obtained. Pure oxalato-chromiates may be prepared by reducing chromic acid with oxalic acid in the presence of sodium oxalate. The compounds which result depend upon the proportion of chromic acid to oxalate employed and upon concentration, temperature, pH value, and the amount of alkali metal present. Werner has described the following classes of oxalato-chromiates:

$3Na^+[Cr(C_2O_4)_3]^{---}$ Sodium trioxalato-chromiate
$Na^+[Cr(H_2O)_2(C_2O_4)_2]^-$ Sodium dioxalato-diaquo-chromiate
$2Na^+[Cr(H_2O)(OH)(C_2O_4)_2]^{--}$ Sodium dioxalato-aquo-hydroxo-chromiate
$3Na^+[Cr(OH)_2(C_2O_4)_2]^{---}$ Sodium dioxalato-hydroxo-chromiate

The hydroxo- compounds may unite to form olated compounds. Thus two molecules of the sodium dioxalato-aquo-hydroxo-chromiate noted above yield the following olated compound:

$$4Na^+\left[(C_2O_4)_2Cr\,\genfrac{}{}{0pt}{}{OH}{HO}\,Cr(C_2O_4)_2\right]^{----}$$

Compounds similar to those noted above may also be formed with acetate tartrate, formate, and other ions.

In 1926,[25] Gustavson tanned hide powder with oxalato-chromiate. This compound was prepared by reducing sodium bichromate with oxalic acid, according to the following equation:

$$Na_2Cr_2O_7 + 7H_2C_2O_4 \longrightarrow 2Na[(H_2O)_2Cr(C_2O_4)_2] + 3H_2O + 6CO_2.$$

A hot saturated solution of oxalic acid was added to a hot solution of bichromate, with continuous stirring. The resulting solution, at completion of reduction, was then boiled for 30 minutes to expel carbon dioxide. Alkali was then added to portions of this stock solution to give a series of basic liquors; this reaction, leading to the formation of hydroxo compounds, may be represented thus:

$$Na\left[(H_2O)_2Cr(C_2O_4)_2\right] + NaOH \longrightarrow Na_2\left[\genfrac{}{}{0pt}{}{H_2O}{HO}\,Cr\cdot(C_2O_4)_2\right] + H_2O$$

It will be recognized that the compound produced is the *cis* form, and this form is presumed, upon aging, to change slowly into the olated compound

already described. Gustavson's solutions were aged for six months before use. Instability of the compound was noted when the liquor's pH value ranged from 8.0 to 8.5.

Two grams of hide powder were agitated for 48 hours at room temperature with 100 ml of the various solutions. Four different chrome concentrations were employed, ranging from 5.0 to 55.0 grams Cr_2O_3 per liter. The pH values of the residual chrome liquors were electrometrically determined. The results of the experiments are shown in Figure 157, where the grams of Cr_2O_3

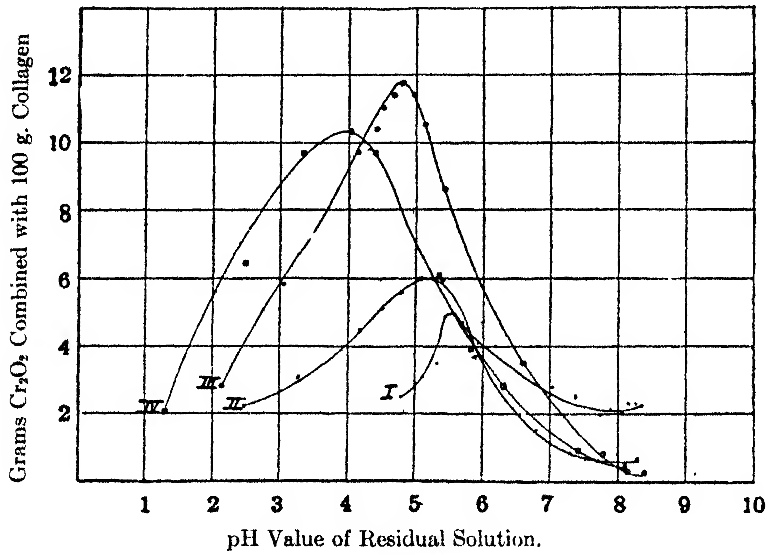


Figure 157. Fixation of chromium by hide powder from solutions of sodium oxalato-chromiate as a function of pH value. Concentration of solutions in grams Cr_2O_3 per liter: I, 5.0; II, 10.0; III, 24.0; IV, 55.0. 2 grams collagen treated with 100 cc solution for 48 hours.

fixed by 100 grams of collagen are plotted against residual pH values. Gustavson did not state whether the tanning period employed was sufficient to ensure the attainment of tanning equilibrium.

It will be noted from Figure 157 that there is, in each concentration series, a maximum chrome fixation which shifts from a pH value of 5.70 to 4.01 as the concentration rises from 5.0 to 55.0 grams of Cr_2O_3 per liter. Gustavson suggested that combination occurs between the anionic chrome and the basic protein groups by means of secondary valence forces, and he stressed that such forces would be at a maximum at the isoelectric point of the protein. This important point, together with the bearing which chromiate tanning has upon our concept of the mechanism of chrome tanning, will be discussed when we consider the theory of chrome tanning.

In unpublished experiments, Theis has studied the tanning behavior of chromiates—sodium dioxalato- and trioxalato-chromiate as well as sodium tetraformato-chromiate. These compounds were prepared in the crystalline form and their electrical migration was entirely anionic. Skin strips were tanned 48 hours with constant agitation at 25°, and then removed. One specimen was pressed twice at 5000 pounds and analyzed, and a duplicate specimen was tested for its shrinkage temperature.

Table 203 shows the result of tanning the equivalent of 5.0 grams of dry strips in 100 ml of liquor containing the amounts of Cr_2O_3 shown, all liquors having been adjusted to a pH value of 5.0.

Table 203. Sodium dioxalato-chromiate.

Gms Cr_2O_3 per 100 ml	Cr_2O_3 Fixed on H.S. (%)	Final liquor pH value	Shrinkage Temp. (° C)
0.25	2.35	4.95	88
0.50	3.34	4.80	93
0.75	4.06	4.60	95
1.00	4.51	4.60	95
1.50	5.36	4.55	99
2.00	6.13	4.55	98

When the pH value of solutions of the sodium dioxalato-chromiate, containing 0.50 grams Cr_2O_3 per 100 ml, was varied by additions of oxalic acid or sodium hydroxide, the results shown in Table 204 were obtained. Exactly similar experiments were then run with the trioxalato with results given in Tables 205 and 206.

The values detailed in these four tables indicate that whereas a considerable degree of tanning occurs when the dioxalato compound is employed, the trioxalato compound has but slight tanning value.

Table 204

Final liquor pH value	Cr_2O_3 Fixed on H S (%)	Shrinkage Temp (° C)
0.90	...	38
1 95	2.16	42
3.02	2.66	71
4.00	3.40	88
4.58	3.52	92
5.41	2.96	92
6.70	1.64	82
7.72	0.92	72
8.52	0.74	70

Table 205

Gms Cr_2O_3 per 100 ml	Cr_2O_3 Fixed on H S (%)	Final liquor pH value	Shrinkage Temp (° C)
0.25	0.51	5.30	62
0.50	0.66	5.10	61
0.75	0.82	5.05	60
1.00	0.98	5.01	60
1.50	1.26	4.90	57
2.00	1.28	4.90	57

Table 206

Final liquor pH value	Cr_2O_3 Fixed on H.S. (%)	Shrinkage Temp. (° C)
1.04	1.50	47
2.00	1.95	51
3.18	1.66	52
4.60	0.78	58
5.20	0.63	60
6.15	0.49	61
6.88	0.42	64
7.38	0.38	67
8.10	0.48	68
8.28	0.51	68
8.80	0.57	68
9.70	0.82	68

The results of employing sodium tetraformato-chromiate in a concentration of 0.50 grams Cr_2O_3 per 100 ml are shown in Table 207. At the completion of the 48-hour tanning period noted, 3.0 ml of formaldehyde (40 per cent) were added to the exhaust tan liquors of a duplicate set and tanning was continued for an additional 24 hours.

Table 207

	No CH_2O added		3.0 ml CH_2O added	
Final liquor pH value	Cr_2O_3 Fixed on H S (%)	Shrinkage Temp. (° C)	Final liquor pH value	Shrinkage Temp (° C)
1.00	0.57	39	1 00	42
2.15	2.45	66	2.05	72
3.00	4.08	87	3.00	91
4.00	6.00	109	3 98	112
4.98	6.81	118	4 91	130
5.28	7.95	122	5.25	133
5.59	7.77	121	5.60	131
6.40	6.67	114	6.67	127
7.90	3.74	90	7.91	116

It will be noted from Table 207 that retannage with added formaldehyde appreciably increases the shrinkage temperature of the leather.

X-ray Examination of Chrome Leather

The numerous studies which have been made of the x-ray diagrams of various leathers are described on pages 602 to 604. These investigations show that chrome tanning changes the diagram of the untanned skin but that when the leather is dechromed the diagram of the untanned skin is completely restored.

pH Values of Chrome Liquors

The pH value of a chrome liquor is changed upon the addition of acid or alkali, or when its free acid is removed by the presence of hide substance. The value varies also as a function of the aging or heating of the liquor and upon the addition of neutral salts, as we have seen; and it must be kept in

mind that basic chrome solutions are buffer systems. The pH value of liquors of the same basicity and concentration may vary widely as a function of their past history. In other words, the pH value of a chrome liquor may not be profitably employed without considering its limitations. When such limitations are kept in mind, however, we can often detect, by means of pH value determinations, changes which may occur in a given tanning system. On the other hand, the tanning behavior of a chrome liquor is not necessarily related directly to its pH value.

Isoelectric Zones of Chrome Leathers

In 1929, Gustavson[26] determined the effect upon the isoelectric point of hide powder when it is tanned with either cationic or anionic chrome compounds. He employed the dye technique described in Chapter 17. Cationic tanning, by means of basic chrome sulfate liquor (containing only cationic chrome), caused the isoelectric point to rise from a pH value of 5.0 to from 6.0 to 7.0. When the powder was tanned with oxalato-chromiate liquor containing only anionic chrome, the pH value dropped to 3.8 to 4.8. These findings could be interpreted to mean that the cationic chrome combined predominantly with acid protein groups, and the anionic with basic groups. But Stiasny[76] has pointed out that such conclusions do not necessarily follow, since if secondary valence combination occurred between the chrome and the carbonyl oxygen of the skin, a shifting of the protein's isoelectric point would result.

In 1941,[93] Theis tanned hide powder with 33.3 per cent basic sulfate (sulfur dioxide-reduced) liquor to which had been added increments of eleven different salts, as shown in Figure 157A. Five grams of hide powder were tanned for 48 hours with constant agitation at 25° in 100 ml of chrome liquor. The tanned powders were then filtered under suction and were thoroughly washed with water, whereby unfixed matter and part of the fixed acid radicals were removed. They were then dried, reground, and their isoelectric points determined by suspending the powders in a buffer solution and employing the Graham vertical cataphoresis cell. The effect of the various salts upon both isoelectric point and chrome fixation are shown in the figure.

We note that all the added anions have shifted the isoelectric point, which Theis believes to be due to a neutralization of collagen amino and carboxyl groups.

Effect of Deaminization

In 1923, Hitchcock[32] deaminized gelatin by treating it with nitrous acid, after which the gelatin contained no amino nitrogen which was detectable by either the Van Slyke or the formol titration methods. Hitchcock then determined the comparative acid-combining capacity of deaminized and

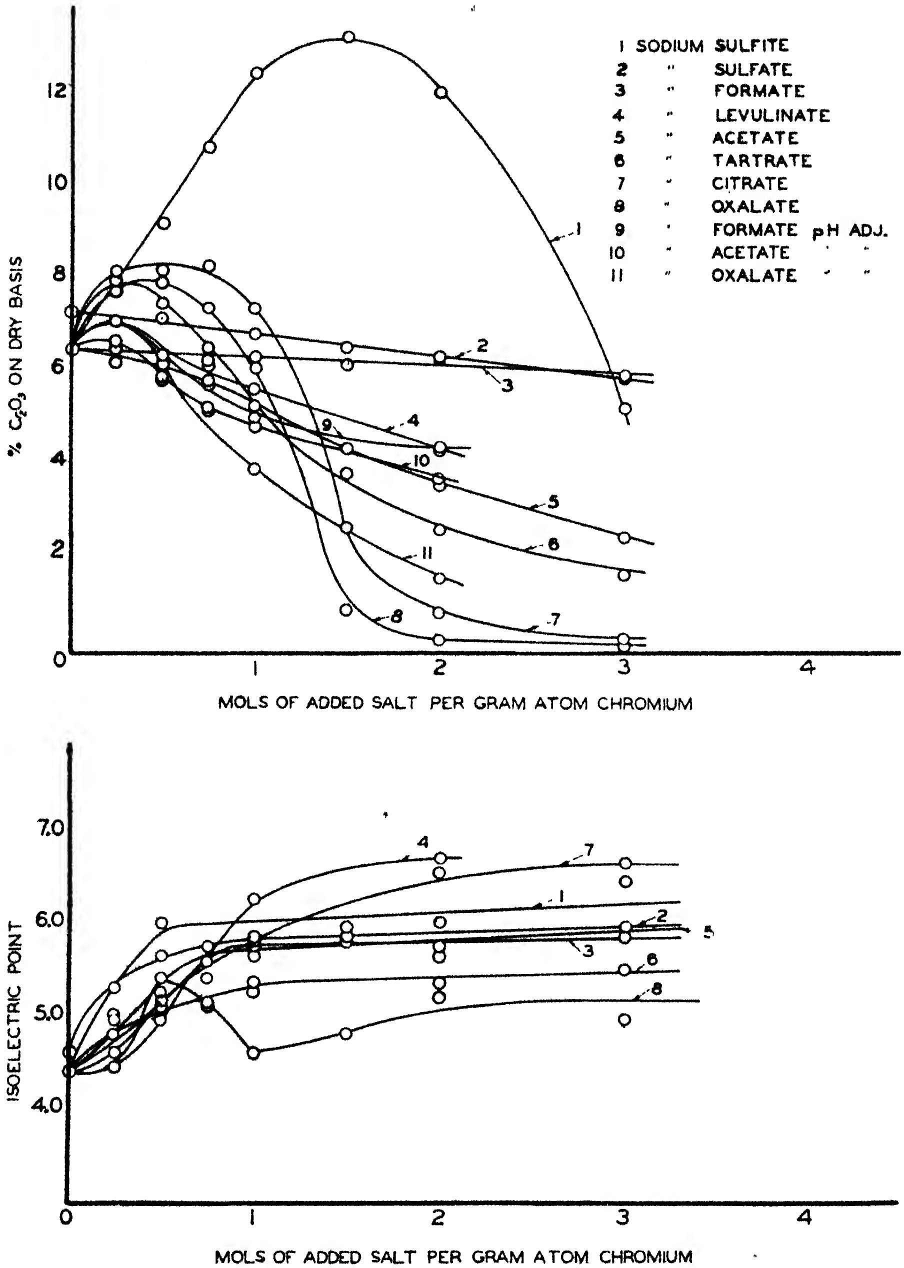


Figure 157A. Data indicating Cr_2O_3 fixation and isoelectric point by addition of various organic salts to a basic chromium sulfate liquor.

untreated gelatin by titration with hydrochloric acid, and found that deaminization had reduced the maximum acid-binding power of the gelatin by about 50.0 per cent.

McLaughlin and Adams, in an unpublished study, have investigated the effect of deaminizing calf skin squares (see page 359) upon their capacity to bind sulfuric acid. This was done by placing 10.0-gram portions of both regular and deaminized squares in 8.0-oz. stoppered bottles and adding sufficient distilled water to hydrate the squares; after 30 minutes the required amount of sulfuric acid was added, together with sufficient water to give a final volume of 100.0 ml. The bottles were then continuously rotated for 24 hours at 19 rpm and at 70° F, this time period having been found to be sufficient for the attainment of equilibrium. At the end of the 24 hours' agitation, the samples were removed, drained and pressed twice in a hydraulic press at a pressure of 5000 lbs per sq in, which removed all uncombined acid. They were then air-dried, ground, and analyzed for nitrogen and fixed acid. The results are shown in Table 208, where all values are on hide substance basis.

Table 208

H_2SO_4 given (%)	Acid fixed by undeaminized hide substance (%) (a)	Acid fixed by deaminized hide substance (%) (b)	(b) ÷ (a)
5.00	4.44	2.50	56
6.00	4.72	2.85	60
8.00	5.38	3.07	57
10.40	5.93	3.50	59
11.20	6 06	3.63	60
12.00	6.08	3.97	65
13.00	6.37	3 99	63
14.00	6.44	4.12	64
15.00	6.46	4.19	65
16.00	6 65	4.22	64
18.00	6.83	4.51	66
19.00	7.00	4.57	65

In 1926, Thomas and Kelly[103] suggested that if the basic groups of collagen play a role in chrome tanning, their removal by deaminization should markedly affect the fixation of chrome. They therefore deaminized standard hide powder according to the method employed by Hitchcock for gelatin. The hide powder before and after deaminization contained 17.91 and 17.64 per cent nitrogen, respectively, on dry basis; this difference in nitrogen content was assumed to be an index of deaminization. Portions of both powders equivalent to 5.00 grams dry basis were tanned for 48 hours (presumably at room temperature) with constant agitation in 200 ml of 52.0 per cent basic chrome sulfate solutions, containing varying concentrations of Cr_2O_3, as shown. The samples were then washed free of uncombined matters and were dried and analyzed; the results are given in Table 209. From their results, Thomas

and Kelly concluded that the basic groups of collagen play a significant role in tanning.

Table 209

Grams Cr_2O_3 per liter	pH original solution	pH exhaust liquor regular hide powder	pH exhaust liquor deaminized hide powder	Cr_2O_3 fixed on H.S. (%) Reg (a)	Deam. (b)	(b) ÷ (a)
3.00	3.32	3.70	3.58	9.00	7.00	77
7.08	3.27	3 38	3.43	16.00	7.90	49
10.90	3.23	3.36	3.37	19.80	8.50	43
15.00	3.17	3.33	3.33	19.50	9.20	47
19.90	3.18	3.29	3.28	17.90	9.40	53
24.90	3.17	3.26	3.24	16.70	8.90	53
34.60	3.13	3.20	3.20	16.30	9.00	55
49.90	3.05	3.13	3.12	15.30	8.90	58
75.30	2.98	3.00	3.02	13.80	7.70	56
125.30	2.92	2.87	2.88	10.20	4.30	42

In 1926 and 1927, Gustavson[25] also studied the effect of deaminization of hide powder upon its power of fixing both cationic and anionic chrome compounds, employing Hitchcock's deaminization method. He tanned 3.00-gram portions of powder with 100 ml of a sodium oxalato-chromiate, containing 12.0 grams Cr_2O_3 per liter for 48 hours, presumably at room temperature. The pH values of the various solutions, which contained a constant amount of chrome, were so adjusted that the values of the exhaust solutions were as shown in Table 210.

Table 210

No.	pH value Exhaust liquor Regular powder	Deaminized powder	Per cent Cr_2O_3 Fixed Regular powder (a)	Deaminized powder (b)	(b) ÷ (a)
1	4.50	4.31	4.96	3 93	79
2	4.68	4.58	5.94	4.50	76
3	4.97	4.75	6.73	5.17	77
4	5.16	5.01	7.09	5.48	77
5	5.45	5.28	6.78	5.02	74
6	5 70	5.52	5.45	4.55	83

Gustavson[27] then tanned both regular and deaminized hide powder with basic chrome chloride and with basic chrome sulfate, employing 2.0-gram portions of dry hide powder and 100.0 ml of chrome solution, for 48 hours, presumably at room temperature. The comparative Cr_2O_3 fixations on hide substance were as follows: 50.0 per cent basic chrome chloride: 20.8 grams Cr_2O_3 per liter, regular 11.05 and deaminized 8.12 per cent; 38.5 per cent basic chrome chloride: 15.2 grams Cr_2O_3 per liter, 8.25 and 6.02 per cent; 59.0 per cent basic chrome sulfate: 18.3 grams Cr_2O_3 per liter, 20.06 and 9.12 per cent; 37.0 per cent basic chrome sulfate: 18.0 grams Cr_2O_3 per liter, 11.89 and 7.38 per cent. In commenting upon his experiments, Gustavson suggested that the decreased fixation from the chromiate solutions by the deaminized powder was a function of the removal of basic groups, indicating that the reaction was

"The formation of molecular compounds between the anionic chrome complexes and the hide protein by means of its basic groups." Gustavson stressed the fact that maximum fixations occurred (see Table 210) at the isoelectric point of the protein, indicating that a primary valence reaction was not involved. In commenting upon his findings with cationic chrome compounds, Gustavson suggested: "The above data evidently favor the view that the inhibition of cationic chrome fixation by hide protein possessing a less number of reactive groups than regular protein is connected with the diminished acid combining capacity of the structurally altered hide powder."

McLaughlin and Adams, in an unpublished study, have investigated the comparative chrome-fixing power of regularly prepared calf skin squares and of squares deaminized after preparation. This was done by tanning 25.0-gram portions of squares in 250 ml of a 35.0 per cent basic chrome sulfate and of the concentrations shown in Table 211. Tanning was for 48 hours at 90° F and with constant agitation at 19 rpm; experiment showed that tanning equilibrium was reached with such treatment. At the end of the tanning period, the squares were drained and were pressed twice at 5000 lbs per sq in, whereby all unfixed chrome was removed. The squares were then dried, ground, and analyzed. The results are given in Table 211, where all results are expressed on hide substance.

Table 211

Cr_2O_3 given (%)	Per cent Cr_2O_3 Fixed: Regular (a)	Per cent Cr_2O_3 Fixed: Deaminized (b)	(b) ÷ (a)
8.20	7.48	6.68	89
10 27	8.72	7.66	88
12.30	9.58	8 28	86
14.38	10.27	8.58	84
16.41	10.92	9.02	83
18.49	11.30	9.20	81
20.55	11.96	9.64	81
22.54	12.35	9.80	79
24.60	12.37	9.98	81

In commenting on the deaminization studies of Thomas and Foster, and of Gustavson, Stiasny[76] points out that since deaminization decreases the fixation of both anionic and cationic chrome compounds, the nature of the charge of the chrome complex cannot be of any decisive importance. Stiasny believes that the real effect of deaminization in lowering chrome fixation is that it changes the protein structurally, decreasing its active surface.

Effect of Various Pretreatments on the Chrome-fixation Capacity of Hide Substance

In addition to the effect of deaminization which has just been described, experimental studies have been made of the effect of various other pretreat-

ments upon the ability of hide substance to fix chrome. The object of such investigations was to throw light upon the mechanism of chrome tanning, more particularly as to which protein groups react with chrome. These investigations will now be described.

Effect of Liming. Gustavson[26] cut 200-gram samples from the butt of a soaked and fleshed cow hide and limed them for varying time periods in saturated lime water containing excess lime. The specimens were then delimed with ammonium chloride, were unhaired and scudded by hand, and

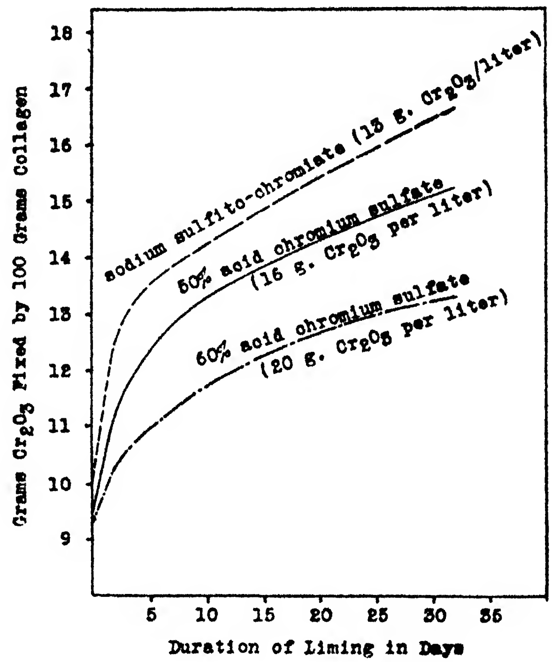


Figure 158. Effect of duration of liming of cow hide upon subsequent fixation of chromium during chrome tanning. 30 grams wet cow hide in contact with 1000 cc. chrome liquor for 48 hours.

were then placed in a two per cent boric acid solution of pH value 5.0. The specimens were well washed before entering the lime solution. Samples from the various liming periods were then tanned in different chrome solutions for 48 hours, together with an unlimed corium control, the flesh and grain layers of which had been mechanically removed. The results are shown in Figure 158, from which it will be noted that large increases in chrome fixation occur as a function of liming time, and in the case of both anionic and cationic chrome compounds. It is not yet possible to decide whether the increased chrome fixations shown are a function of a chemical change in the hide sub-

stance, or of physical change, thereby increasing active surface, or whether both effects are involved.

Effect of Pretreatment with Enzymes. Since hides and skins are usually bated with tryptic enzymes before chrome tanning, Gustavson[26] has made a preliminary investigation of the effect of enzymatic action upon subsequent chrome tanning. This was done by treating standard hide powder for five hours at 25° with 0.2 per cent strength pepsin in a citrate-phosphate buffer of pH value 2.2; other hide powder portions were treated for 5 hours at 40° with a 0.1 per cent strength trypsin in a phosphate buffer solution of pH 8.0. The activity of the enzymes is not stated. The treated powders were then washed, dehydrated with acetone, and dried. Two-gram portions of the various powders were then soaked in 20 ml of distilled water for 6 hours and then for 60 hours in the chrome solutions employed. They were then washed free of uncombined matter and were dried and analyzed. The results are shown in Table 212.

Table 212

Pretreatment	Grams Cr_2O_3 Fixed per 100 grams Collagen: 37.0% Basic chrome sulfate (20 0g Cr_2O_3 per l)	59 0% Basic chrome sulfate (12 5g Cr_2O_3 per l)
None	12.56	22.56
Trypsin at pH = 8.0	12.00	18.70
Blank at pH = 8 0	12 85	24.72
Pepsin at pH = 2.2	13.91	27.05
Blank at pH = 2.2	13.34	28 32

Gustavson suggested that the findings in this preliminary study may possibly be explained as a removal of activated protein groups, thus lessening the capacity of the protein to fix chrome. In view of the importance of the bating process in chrome tanning, it is to be hoped that these preliminary studies will be greatly extended, employing pieces of variously bated skin, rather than hide powder, and making sure that tanning equilibrium is reached in all cases.

Effect of Pretreatment with Formaldehyde. In 1921, Gerngross[16] stated that pretanning hide powder with formaldehyde lessened its power to fix cationic chrome; and in 1922, Griliches[17] stated that such pretreatment had little or no effect on cationic chrome fixation. But the experimental procedures of both these workers were not such as to insure highly accurate results. In 1927, Gustavson[28] tanned hide powder with varying concentrations of formaldehyde, and at pH values ranging from 2.4 to 12.2, by rotating 5.0 grams of hide powder with 100 ml of formaldehyde solution for 48 hours. The treated powders were then washed free of uncombined formaldehyde, after which they were tanned in the several chrome solutions employed; they were again washed, and were dried and analyzed. No determinations of fixed formaldehyde before chrome tannage were made.

The results may be summarized as follows: The pH value of the formaldehyde pretannage greatly affects the behavior of the powder in subsequent chrome tannage. Pretannage at pH values of 6.0 and 8.0 decreases the fixation of Cr_2O_3 from both basic sulfate and chloride solutions; fixations of anionic sulfito and oxalato compounds are also decreased. Pretannage at the very high pH value of around 12.0 led to increased fixation from cationic liquors, increased fixation from anionic sulfito liquors, but decreased fixation from anionic oxalato liquors. Gustavson offered the following explanations for these results: (1) the diminished fixation of cationic chromium was probably caused by the decreased ability of the pretanned powder to fix the hydrolyzed acid of the chrome solution, since the formaldehyde partially inactivated the protein basic groups; (2) the inactivation of basic groups also reduced anionic chrome fixation, since such chrome, according to his view, combines with basic groups; (3) the increased cationic chrome fixation induced by pretannage at high pH values is probably a function of the activation of carboxyl groups or the breaking up of the internal protein structure by the action of the alkali which was added to the formaldehyde solutions in raising their pH values; and (4) the increased Cr_2O_3 fixation from anionic sulfito solutions, in the case of the formaldehyde tannages at high pH values, is probably related to deaggregation of the protein structure by the added alkali.

In view of our newer and more complete understanding of formaldehyde tanning (see Chapter 12), it is to be hoped that further work will be done regarding the effect of formaldehyde pretannage on subsequent chrome fixation. But such experiments should be performed with skin pieces rather than hide powder, and the uncombined formaldehyde should be removed by pressing and the fixed formaldehyde determined both before and after chrome tanning. In this way it may be possible to secure quantitative relationships between the various reactions which are involved and thus gain fundamental information. Care should be taken that all chrome tannages are run to true equilibrium.

Effect of Pretannage with Oppositely Charged Chrome. In 1927, Gustavson[29] suggested that if cationic and anionic chrome compounds combine with different protein groups, pretannage and then retannage with compounds of opposite charge might throw light upon the tanning mechanism. He therefore tanned standard hide powder with cationic chrome, washed out uncombined matter (together with part of the bound acid) and then retanned the powder with anionic compounds. This process was also reversed, by tanning with anionic chrome and then retanning with cationic. The results indicated that in some cases one type of tannage was independent of the other; but the opposite was found in other cases, and hence no final conclusions may be drawn from the experiments. Gustavson suggested that in cationic tanning a

simultaneous and interdependent double reaction occurs, whereby the chrome combines by means of primary valence with the protein carboxyl groups, while the hydrolyzed acid of the chrome solution combines with basic protein groups; but that anionic chrome, on the other hand, forms molecular compounds with protein basic groups.

According to this view, therefore, if hide substance is completely tanned with cationic chrome (so that all its available carboxyl groups combine with chrome and all available basic groups with acid) no fixation of anionic chrome could occur unless the anionic chrome displaced the combined acid and thus made basic groups available for reaction. Or, if hide substance is completely tanned with anionic chrome (whereby all available basic groups are inactivated) no cationic fixation could occur unless the hydrolyzed acid of the cationic chrome solution displaced the fixed anionic chrome from the basic groups.

This subject is of sufficient importance to merit further investigation. But we recommend that skin pieces rather than hide powder be employed; that complete tannage be attained in all cases; and that the leather be pressed rather than washed, so that combined acid radicals may be accurately determined.

Pretreatment with Quinone and with Vegetable Tannins. Thomas and Kelly[103] tanned standard hide powder with quinone and also with wattle tannin. The tanned powders were then washed free of soluble matter and were air-dried and analyzed. Portions were then tanned with increasing concentrations of 33.0 per cent basic chrome sulfate, after which they were washed, dried and analyzed. The results showed that pretannage with either quinone or wattle tannin greatly decreased cationic chrome fixation. This led Thomas and Kelly to assume that the inactivation of basic protein groups (by their combination with quinone or tannin) inhibited the fixation of cationic chrome. Gustavson reversed the procedure; that is, he first tanned hide powder with either cationic or anionic chrome and then retanned it with vegetable tannin. He found the cationic pretanned powder to fix more vegetable tannin than the unchromed control, whereas the anionic pretanned powder fixed less.

The experimental results described above are confusing, since no clear-cut relationships are apparent. Thomas and Kelly ascribed their results to an inactivation of basic protein groups; Gustavson considered that cationic chrome combination with protein acidic groups would activate basic groups, rendering them reactive. This subject has been discussed at length in Chapter 17, in connection with the effect of chrome tanning upon subsequent tannin fixation and, also, in connection with the effect of vegetable tanning on the acid-combining capacity of hide substance. The later studies of Page and Otin and Alexa have there been discussed in these connections.

Comparative Distribution of Fixed Chrome in Various Skin Layers

Chrome tanners have long known that the uniformity of chrome fixation throughout the various layers of skin is a function of the nature of both the pickling and the subsequent one-bath tanning process. One of the distinguishing characteristics of certain Continental upper leathers, for example, is the fact that the fixed chrome they contain is uniformly distributed throughout their grain, middle and flesh "layers." This even chrome distribution is desirable in some types of leather, but it is not necessary, or even desirable, in other types. On the other hand, a too great inequality of chrome fixation often induces undesirable leather qualities. In 1909, Procter and Law[65] showed that the hydrolyzed acid of a chrome liquor diffused into gelatin jelly at a much faster rate than did the basic chrome compound produced by such hydrolysis. Such diffusion differentials lead to an excessive chrome deposition in the surface layers of the skin. The prevention of such excessive deposition is one of the principal objects of pickling; and one of the functions of masking compounds in the one-bath process is retardation of the tanning rate.

The first publication regarding the subject under discussion was that of R. F. Innes,[37] in 1914. Innes called attention to the importance of determining fixed chrome in the various skin layers In 1929, Schindler and Klanfer[72] studied the effect upon chrome fixation of variations in pickle composition, liquor basicity and liquor aging, precipitation figure, temperature of tanning, etc. in the tanning of calf skins.

More recent studies of this problem have been those of Theis and his collaborators,[94] and of Gustavson.[30] The former authors tanned bated steer hide for six hours with basic chrome sulfate liquors of basicities ranging from zero to 53.0 per cent, with and without the addition of sodium sulfate to the chrome liquors. After tanning, the specimens were washed and dried and were then split into grain, middle and flesh layers which were analyzed for fixed Cr_2O_3. Chrome fixations were very non-uniform in the case of all basicities when no added sodium sulfate was present. For example, in the case of the 33.0 per cent basic liquor, containing no added salt, the middle layer fixed only 0.20 per cent Cr_2O_3 (hide substance basis), whereas the total fixation for all three layers was 2.07 per cent. But when the same liquor was made to contain 2.0 moles of added sodium sulfate per mole of basic chrome sulfate, the middle layer fixed 3.94 per cent Cr_2O_3 and the total fixation for all three layers was 3.60 per cent. These authors explained the action of the added sodium sulfate as a function of its effect upon the chrome compound; that is, sulfate radicals entered the complexes and also changed their molecular aggregation. In discussing the experiments just described Gustavson attributed the non-uniform findings noted to be a function of the swollen condition of the hide specimens (which condition prevented the free inward diffusion of the chrome liquor). He does not believe the findings are

to be explained as a function of change in the chrome compound induced by added sodium sulfate.

In a later study, Theis[95] showed that, although the contentions of Gustavson are correct in respect to the influence of skin swelling upon uniform chrome fixation, this is not the complete explanation. This is shown by the fact that even though all swelling is prevented by the use of an adequate pickle, inequalities of chrome fixation may result in the absence of sodium sulfate except when sufficient salt is present *in the chrome liquor*. For this reason, he believes that one of the principal functions of the added sulfate is the effect upon the chrome compound.

Holland[33] tanned pieces of calf skin, which had been pickled with hydrochloric acid and sodium chloride, with 33.0 per cent basic chrome sulfate, to which was added 0.25 or 0.50 mole of various sodium salts per mole of $Cr(OH)SO_4$. No description of the tanning method is given, but it is stated that the various specimens were washed in running water for 30 minutes at the end of tannage. They were then split into grain, middle and flesh and the splits were analyzed for fixed chrome. The results may be summarized as follows: (1) the control splits showed no appreciable difference in fixation, nor did those in which the chrome liquor contained the following additions: 0.25 mole sodium bicarbonate, 0.50 mole bicarbonate, 0.50 mole formate, 0.50 mole acetate, or 0.50 mole oxalate; (2) those receiving 0.25 or 0.50 mole of succinate, adipate and phthalate all showed inequalities of fixation in the three splits.

Reversibility of Chrome Tanning

In 1916, Procter and Wilson[64] showed that if skin which had been tanned with cationic chrome compounds was subjected to the action of a solution of Rochelle salt (or similar salts) the fixed chrome it contained was completely removed. They found that such dechromed skin, from which all reversing salt was removed by washing, could again be chrome-tanned; the chrome fixation was, in other words, a completely reversible process. This original observation was followed by numerous investigations of other workers, in connection, more particularly, with the dechroming of chrome leather scraps for use in the manufacture of glue. Among these more recent studies may be mentioned those of Bennett,[7] Berestovoj and Masner,[8] and Simoncini. The latest and most inclusive investigation is that of Lollar.[48] Lollar has extensively studied the reversal of fixed chrome by means of various acids, alkalies, and salts. He has given an interesting discussion of the mechanism of the reaction between such compounds and the deposited or fixed insoluble 66.7 per cent basic chrome sulfate (in the case of sulfate tannage), whereby the latter is converted into a soluble, non-tanning chrome compound.

In this section we are, however, more specifically concerned with whether

chrome tanning is, or is not, a reversible reaction in the absence of added "foreign" ions, as described in the preceding paragraph. The importance of this question is found in its very direct bearing upon our theoretical approach to the mechanism of chrome tanning. Except for those cases of chrome reversal by means of foreign ions or by drastic dechroming methods, the very general assumption until recently has been that we deal in chrome tanning with an almost completely irreversible reaction. This assumption has been due to the fact that we may indefinitely wash chrome leather in *running water* without removing any of its fixed chrome. We now know that this merely reflects the fact that deposited or fixed chrome is quite insoluble in water. But when chrome leather, which has been tanned with basic chrome sulfate, is appropriately treated in a *closed system*, it is found that the fixed chrome is completely reversible. We shall now discuss the experimental evidence of these statements.

In 1937, Cameron, McLaughlin and Adams,[12] in a preliminary investigation, studied the extent of fixed chrome reversal when calf-upper leather was treated with successive portions of a mixed solution of sulfuric acid and sodium sulfate. (The sodium sulfate was added to prevent the swelling of the leather as it becomes partially dechromed; if the leather is allowed to swell, the outward diffusion of reversed and soluble chrome is inhibited.) The leather was pressed after tanning, whereby all unfixed chrome and unfixed acid were removed, and was then dried at room temperature for 168 hours; it contained 11.35 per cent fixed Cr_2O_3 and showed a basicity of 50.0 per cent. This dry leather was then treated with successive portions of the acid/salt solution at room temperature and the treatment removed 98.57 per cent of the original fixed chrome. Treatment with Rochelle salt removed 98.09 per cent. When dry leather containing 8.00 per cent fixed Cr_2O_3 was treated (both before and after aging for three weeks at room temperature) with oxalic acid and then with sodium oxalate, all the fixed chrome was removed in both cases. These authors then proceeded as follows.

Portions of skin squares were tanned to equilibrium with varying basicities of chrome sulfate and after pressing showed the percentages of fixed Cr_2O_3 and the basicities given under (a) in Table 213. Sufficient duplicate portions had been tanned to contain 11.40 per cent fixed Cr_2O_3 to permit demonstration of the following assumption: If chrome fixation is reversible, then retanning the control squares (containing 11.40 per cent fixed Cr_2O_3) in their exhaust liquors of lowered basicities should cause them to lose fixed chrome. The duplicate portions mentioned were drained after tannage and their exhaust liquors were retained. The tanned squares (containing 11.40 per cent fixed chrome) were pressed twice at 5000 pounds and then kept in sealed jars and were allowed to age before retannage for the time periods noted in the table. (The experiment shown under (f) represents tanned squares which were dried

in the open air and were not aged in closed jars.) The object of aging before retannage was to determine the effect of aging upon reversibility.

At the end of the various aging periods noted, sufficient sulfuric acid was added to the various exhaust liquors to lower their overall basicity to 36.8, 12.0 and −3.7 per cent basicity, respectively. That is, such overall basicities were based upon all the chrome and acid contained in the pressed leather (11.40 per cent fixed chrome and 11.22 per cent fixed acid SO_4) and all the chrome and acid in the exhaust liquors. The tanned squares were then put back into the exhaust liquors whose basicities had been lowered and were retanned for 48 hours at 90° F and with constant agitation at 19 rpm. The squares were then pressed twice at 5000 pounds, dried, and analyzed. The results are shown in Table 213, from which we note: (1) Previously fixed

Table 213. Showing Both Reversibility of Chrome Tannage and that Leather Attains the Same Basicity as that of Liquor, at Equilibrium.

Control (a)			No Aging (b)			Aged 24 hours (c)		
% Cr_2O_3 Fixed	% Acid SO_4 Fixed	Basicity	% Cr_2O_3 Fixed	% Acid SO_4 Fixed	Basicity	% Cr_2O_3 Fixed	% Acid SO_4 Fixed	Basicity
11.40	11.22	48.0	11.40	11.22	48.0	11.40	11.22	48.0
10.13	12.12	36.8	10.09	12.45	34.9	10.01	12.05	36.4
7.60	12.66	12.0	7.70	13.05	11.4	7.74	13.09	10.7
6.46	12.70	−3.7	6.83	13.06	−1.0	6.96	13.67	−3.7

Aged 168 hours (d)			Aged 296 hours (e)			Air-dried 168 hours (f)		
% Cr_2O_3 Fixed	% Acid SO_4 Fixed	Basicity	% Cr_2O_3 Fixed	% Acid SO_4 Fixed	Basicity	% Cr_2O_3 Fixed	% Acid SO_4 Fixed	Basicity
11.40	11.22	48.0	11.40	11.22	48.0	11.40	11.22	48.0
10.45	12.35	37.6	10.25	12.20	37.8	10.06	12.39	35.0
7.92	13.02	13.2	7.90	12.71	15.0	7.55	13.15	8.0
7.08	13.13	3.0	7.04	13.09	1.8	6.60	13.37	−7.0

chrome has been reversed until it has reached the values of the controls shown in (a); (2) with the exception of the −3.7 per cent basic experiments, the retanned leathers show essentially the same basicities as the controls; and (3) the aging treatments given have not affected reversibility.

It was next shown that the opposite of the reversals noted in Table 213 may be obtained. This was done by tanning five portions of squares with 16.28 per cent Cr_2O_3 (on hide substance) of a 1.00 per cent basic chrome sulfate liquor for 48 hours at 90° F and with agitation at 19 rpm. At the end of tannage a sample of the squares was pressed, dried, and analyzed and showed the chrome fixation and the basicity of the control in Table 214. Sodium hydroxide was then slowly added to the four exhaust liquors of the duplicate portions to produce *overall* basicities of 10.00, 20.00, 30.00 and 40.00 per cent basicity, respectively. The tanned and pressed but undried squares were then put back into the exhaust liquors and were retanned for 48 hours

at 90° F with constant agitation at 19 rpm, after which they were pressed, dried and analyzed. The results are given in Table 214.

Table 214

Number	Cr_2O_3 Fixed (%)	Acid SO_4 Fixed (%)	Leather Basicity	Overall Basicity Given
1 (Control)	5.64	10.63	−00.4	1.00
2	6.69	11.35	10.4	10.00
3	7.89	12.00	19.7	20.00
4	9.28	12.06	31.3	30.00
5	10.62	12.15	39.6	40.00

The various experiments which have just been described show that hide substance attempts to come into equilibrium with its environment in chrome tanning. In so doing, it may either lose or gain fixed chrome and its overall basicity will be that of the overall basicity of the system in which it is tanned. This is assuming, of course, that tanning conditions are such as to insure the attainment of true equilibrium.

McLaughlin and Cameron[53] next demonstrated that fixed chrome may be reversed by mere dilution of the system in which the hide substance was tanned. In other words, reversal occurs without the addition of any ions. Twenty 5-gram portions of skin squares were tanned in 500 ml of basic chrome sulfate liquors, of the basicities shown in Table 215, for 48 hours at 90° F with constant agitation at 19 rpm. Two duplicate sets of samples were tanned in the same manner and with the same liquors, except that the liquor volume was 250 ml instead of 500 ml. At the end of tannage one set was pressed and analyzed. To the other set was added 250 ml of distilled water (bringing the final volume to 500 ml) and tannage was continued for 48 hours.

Table 215

	(A) Control, 500 ml		(B) 250 ml		(C) (B) + 250 ml. H_2O	
Basicity of Liquor given	Cr_2O_3 fixed (%)	pH Exhaust liquor	Cr_2O_3 fixed (%)	pH Exhaust liquor	Cr_2O_3 fixed (%)	pH Exhaust liquor
48.8	12.46	3.6	12.80	3.6	12.42	3.6
38.5	10.75	3.3	11.36	3.3	10.56	3.3
35.9	10.00	3.1	10.50	3.1	9.93	3.1
28.6	9.42	2.9	9 88	2.9	9.30	2.9
18.9	7.88	2.4	8.80	2.3	7.72	2.4
8.8	6.60	2.3	7.18	2.2	6.54	2.3
−0.5	5.24	2.2	5.82	2.0	5.36	2.2

We note that whereas the higher concentration, (B), caused greater chrome fixations than in the case of the lower concentration, (A), that when the concentration of (B) was lowered to that of (A), fixed chrome was reversed until the fixations of (A) and (C) were practically identical. The actual amount of Cr_2O_3 given was the same in (A), (B) and (C); the concentration was varied.

These authors then demonstrated that if pressed chrome leather, which

contains no unfixed chrome or acid, is placed in water to which raw skin has been added and the mixture is agitated, both fixed chrome and fixed acid will leave the leather and will combine with the added raw skin. Portions of skin squares were tanned for 48 hours at 90° F with constant agitation at 19 rpm and were then pressed twice at 5000 pounds. One part of each portion was then dried and analyzed for fixed chrome and leather basicity, as shown in Table 216. Another part of the pressed leather, containing about 50.0 per cent moisture, and representing 25.0 grams of hide substance, was added to 500 ml of a 1.6 per cent sodium sulfate solution together with 25.0 grams of marked untanned skin squares; all were in a closed jar. The various jars were then agitated for six weeks at 90° F and at 19 rpm. (The sodium sulfate was added to prevent swelling of the added raw skin.) At the end of six weeks' agitation the specimens were removed, were pressed twice at 5000 pounds, and then dried and analyzed. The results are shown in Table 216,

Table 216. Variation of Amount of Original Chrome Fixed and of Leather Basicities.

No.	% Fixed Cr_2O_3 Present	Basicity of Leather	A % Fixed Cr_2O_3 of Leather After 6 Weeks Drumming	B % Fixed Cr_2O_3 of Added H.S After 6 Weeks Drumming	pH of Solution After 6 Weeks Drumming	Percentage of Theoretical Reversal
1	15.50	49.4	11.00	4.32	4.1	56.4
2	13.80	49.4	10.20	2.97	4.3	45.1
3	11.20	49.4	9.35	1.90	4.5	33.8
4	12.52	34.6	6.96	5.19	3.7	85.4
5	10.25	34.6	6.95	3.14	4.0	62.2
6	7.30	34.6	5.55	1.55	4.4	43.7
7	7.54	18.0	4.92	2.59	3.7	69.0
8	6.06	18.0	4.27	1.50	4.1	52.0
9	4.21	18.0	3.32	0.71	4.6	35.2

the last column of which shows the "percentage of theoretical reversal." This figure is derived by adding together the fixed chrome of the A and B columns and dividing by two in order to ascertain the percentage of fixed Cr_2O_3 which each type of hide substance should contain if a complete sharing of chrome between the two samples had occurred. The figure thus derived is divided into the percentage of fixed Cr_2O_3 actually present in the added and formerly raw hide substance, yielding the figures in the last column of the table.

The interesting results shown in Table 216 may be explained as follows. If tanned and pressed leather is agitated with water in a closed system, a small amount of bound acid is hydrolyzed, moving out into the surrounding solution and dissolving a trace of fixed chrome. Equilibrium is soon attained, however, and no more chrome and acid are reversed; but the addition of raw hide substance continually disturbs the equilibrium and permits the significant reversals shown. It will be noted that the extent of reversal appears to be a function of the pH value of the solution, as would be expected. If the added

raw hide substance is pickled with sulfuric acid, the pH value of the solution is further decreased, and reversals approximating 100 per cent of theoretical are obtained.

In order to ascertain the effect of long aging of leather upon the reversal of its fixed chrome, McLaughlin and Adams[54] tanned skin squares with basic chrome sulfate liquor so that they would contain the fixed chrome and basicities after pressing that are shown in Table 217. Portions of the pressed, undried leather were then placed in sealed jars and were held for the time periods noted. They were then agitated for six weeks in a 1.6 per cent solution of sodium sulfate containing an equal weight of marked raw skin squares (dry basis). At the end of six weeks' agitation the various samples were pressed twice at 5000 pounds and were dried and analyzed. The reversal data given in Table 217 indicate that aging pressed (undried) leather decreases the reversibility of its fixed chrome; the extent of such decrease appears to be reached during six months of aging.

Table 217. Aging Leather Before Reversal.

No	% Fixed Cr_2O_3 Present	Basicity of Leather	Weeks Aged Before Reversal	A % Fixed Cr_2O_3 of Leather After 6 Weeks Drumming	B % Fixed Cr_2O_3 of Added H S After 6 Weeks Drumming	pH of Solution After 6 Weeks Drumming	Percentage of Theoretical Reversal
1	15.50	49 4	0	10 80	4.28	4.1	56 8
2	15.50	49 4	1	11.65	3 77	4.1	48.9
3	15 50	49.4	2	11.86	3.48	4.2	45.4
4	15.50	49.4	4	12.23	3.04	4 1	39.8
5	15 30	48.2	0	10.75	4.75	4 1	61.2
6	15.30	48.2	26	12.22	2 85	4.0	37 8
7	15.30	48.2	52	13.03	2 74	4.1	34 7
8	8.78	22 8	0	5 06	3.39	3 6	80.2
9	8 78	22.8	26	5.51	2.43	3.5	61 2
10	8.78	22 8	52	5.90	2 46	3 8	58.8

We have now seen that cationic chrome fixation is a reversible process It is to be hoped that reversal investigations will be conducted with anionic fixations.

It has long been known that all the combined acid radical present in cationic-tanned chrome leather may be reversed and removed by subjecting the leather to the hydrolyzing action of running water. Wilson and Lines[108] demonstrated this in 1926. The length of washing treatment which is required for complete removal of acid radical varies with the composition of the leather. No cationic fixed chrome is removed by running water.

Thermolability of Chrome Leather

As has been noted, the "boil test" has long been employed by chrome tanners as a rough test of degree of tannage. The unusual behavior of chrome leather toward heat constitutes a problem which has engaged the attention of many workers, and we shall now discuss their investigations and interpre-

tations. The phenomenon has an important bearing upon the theory of chrome tanning.

In 1905, Korner[40] explained the stability of chrome leather toward hot water as a function of the chrome-tanning agent forming an insoluble solid solution throughout the skin fiber.

In 1927, Hudson[35] discussed the phenomenon and suggested that the nature of the acid radicals of the fixed chrome compound largely determined the heat stability of the leather. He suggested that monovalent radicals lower such stability, whereas divalent radicals increase it. He found that lowered heat stability of the leather resulted when part of the divalent sulfate radical fixed by skin in chrome sulfate tanning was presumably replaced by the monovalent chloride radical. Hudson postulated two types of combination in basic chrome sulfate tanning: (1) A leather compound in which the Cr atom is attached to protein carboxyl groups by primary valence, and (2) a compound in which the Cr atom is indirectly attached to the protein amino groups, by means of the divalent acid radical of the chrome compound. He found that if sulfate-tanned leather was treated with 5.0 per cent sodium chloride solution for 4.0 hours, the second type of chrome fixation was reduced, as was also the heat stability of the leather. But if the sodium chloride was washed out of the leather and it was then treated with 5.0 per cent potassium sulfate solution, the heat stability returned to the value of the control. Gustavson, in commenting on the hypothesis of Hudson, pointed out that whereas thiocyanate and formate ions will displace fixed sulfate groups from chrome leather, they increase the heat stability of such leather.

In 1932, Elod and Siegmund[14] subjected basic chrome sulfate-tanned leather, containing increasing percentages of fixed Cr_2O_3 (starting at 5.72 per cent), to electrodialysis, whereby all sulfate radical was removed. The several specimens were then subjected to the action of boiling water and showed no shrinkage in area. Küntzel and Riess[45] state they find just the opposite of these results, although they give no experimental data to support their contention. McLaughlin and Adams, in unpublished investigations, have studied the heat stability of basic chrome sulfate-tanned calf skin leather containing a constant amount of fixed Cr_2O_3 (4.75 per cent on hide substance) and of varying basicity. The bends of tanned (unneutralized) calf skins were cut into a number of portions and duplicate specimens were

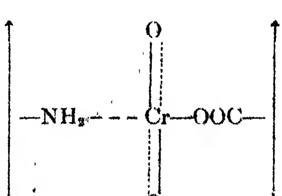
Table 218

Hours Washed	Basicity	Shrinkage (%)
0	40.4	40.0
4	45.2	40.0
24	51.7	19.0
96	70.0	2.0
168	86.4	15.0
336	94.6	29.0

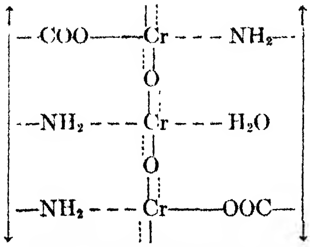
then washed in running water for the time periods shown. At the end of the washing period the specimens were removed; one was pressed and analyzed and the other was subjected to the action of boiling water for one minute and the area shrinkage was measured (Table 218).

These figures indicate that the heat stability of the leather employed is a function of basicity; the leather approaches a minimum shrinkage in the neighborhood of 66.7 per cent basicity (at which the bulk of protein-bound acid has been removed) and then rises as basicity increases. But these experiments must be greatly extended, employing leathers of increasing fixed chrome content, before we can be sure that this finding is of general application. In 1935, Lloyd[47] suggested that chrome tanning destroys the hydrophilic properties of collagen, inactivating the active centers of the protein molecule, and that this leads to loss of chemical activity, to loss of water, and to protection of the peptide links.

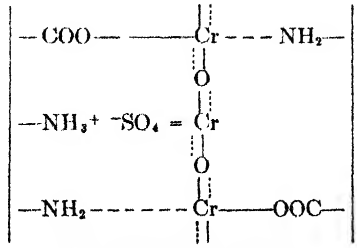
In 1936, Küntzel and Riess[45] discussed the subject of heat stability of chrome leather. They suggested that the reason chrome leather tanned with basic chrome sulfate is more heat-stable than that tanned with basic chloride or nitrate is because of the greater ability of the sulfate radical to enter the coordination sphere of the chrome complex. They stated that leather tanned with basic chrome chloride, which shrank in boiling water, became heat-stable if treated with solutions of any of the following sodium salts: sulfate, bisulfite, thiosulfate, formate, and chromate, or with potassium dioxalato-aquo-hydroxo-chromiate. (No descriptions of experimental methods are given, nor are any supporting experimental data shown.) Küntzel and Riess speculate that complexly bound sulfate radicals do not increase the heat stability of leather by forming bridges between two chromium atoms, thus strengthening the aggregations of the tanning particles. They consider it more likely that the sulfate radical is complexly bound to a chromium atom, giving it a negative charge. The resulting anionic compound then combines with a free protein basic group, forming an un-ionized salt. In other words, they picture the formation of what might be termed an amphoteric tanning compound which will react with the acid groups of one protein chain and with the basic groups of another. In this way the protein chains will be riveted together, imparting heat stability to the leather. They picture the combination between the negatively charged chrome and the protein basic groups as follows:



and they suggest the following speculative formulas to describe the constitution of chrome leather which is not heat-stable (I), and that which is heat-stable (II).



I. Not resistant to boiling



II. Resistant to boiling

In 1937, Gustavson[31] reported a very extensive series of experimental investigations of the heat stability of chrome leather and of various factors which influence it. These studies may be summarized as follows. The experiments were conducted with chrome-tanned calf leathers. The leather was put directly into boiling water for three minutes and its area shrinkage after the boiling treatment was measured, with the following results. (1) When calf skin was subjected to liming for a period up to 28 days, and was then chrome-tanned, thermal stability was reduced, which Gustavson attributed to extensive changes in and loosening of the skin structure by the liming. (2) Delimed calf skin was treated with hydrochloric acid solution (pH = 1.0) and also with sodium hydroxide (pH = 12.0) and was then brought to pH = 5.0, was washed free of inorganic constituents, and was then chrome-tanned. When the tanned specimens thus pretreated were examined for thermal stability it was found that treatment with the acid solution had decreased this property, whereas the alkaline treatment had increased it. (3) When specimens were pretanned with formaldehyde or with vegetable tannin or syntan, only the latter treatment caused a change in thermal stability, markedly lowering it. (4) Chrome-tanned leathers which were not resistant to the action of boiling water could be rendered so by retanning with formaldehyde. (5) The action of various salt solutions on skin which was fully tanned with chrome chloride (but which was not thermally stable) indicated that those salts capable of complex formation (especially those which reacted alkaline) exerted a stabilizing influence at low concentrations. At higher concentrations they exerted a detanning action and reduced stability. Sodium sulfate solutions increased stability, but potassium nitrate reduced it. High concentrations of sodium chloride reduced heat shrinkage. On the other hand, treatment with sodium chloride reduced the stability of leather tanned with chrome sulfate. (6) Shrinkage of skin tanned with chrome chloride was completely eliminated by treatment with sodium sulfate,

and this thermal stability remained even when the treated leather was thoroughly washed before the boil test. Treatment with sodium chloride also eliminated the shrinkage, but increased it if the sodium chloride was washed out of the treated leather. (7) Leather tanned with 33 per cent basic chrome sulfate, and containing 8.2 per cent Cr_2O_3 on hide substance, was treated with various salt solutions for 24 hours before boiling. The control showed no shrinkage when boiled, but considerable shrinkage occurred after treatment with all the various salts except sodium sulfate. (8) Leather was tanned with a solution prepared from a dry basic chrome sulfate extract to contain 5.2 per cent fixed Cr_2O_3. This leather shrank 48 per cent in area when boiled; but if first soaked for 72 hours in a $0.25N$ $NaSO_4$ solution, it showed zero shrinkage on boiling.

Gustavson concludes from these studies that thermal stability is a function of the presence of combinations of structural units in the Cr-collagen complex The chrome complexes serve as bridges between ionic-covalent forces and the acid protein groups of one chain and between the coordinative valencies and the uncharged basic groups of another peptide chain. Thus the type of chrome salt, and especially the kind and number of its acid groups (and their stability), are the important factors in determining the thermal stability of chrome leather. These conclusions, it will be noted, are essentially the same as those reached by Küntzel and Riess; all three authors have thus accepted and applied the original riveting or bridging suggestion of Spiers, which will be discussed in the next chapter.

McLaughlin and Adams, in an unpublished study, have treated calfskin leather, which was tanned with basic chrome sulfate to contain 4.5 per cent Cr_2O_3, with sodium sulfate solutions of varying concentration. This was done by cutting specimens from the bends of the tanned skins, washing them in cold water for 20 minutes, and then soaking 1 part of wet leather in 10 parts of sodium sulfate solution for 72 hours at 70° F, after which they were removed, placed in boiling water for 5 minutes, and then measured. The washed control shrank 45 per cent in area, that soaked in $0.25N$ salt 46 per cent, $1.00N$, 42 per cent, and $2.00N$, 43 per cent. The experiment was repeated, employing whole tanned skins and a much larger volume ratio of salt solutions, with essentially similar shrinkage results. These findings are just the opposite of those of Gustavson; but it may be that differences in leather basicity, or in experimental procedure, or the fact that Gustavson employed a dry tanning extract whereas McLaughlin and Adams used a regular glucose-reduced liquor, may account for the divergence.

In a series of papers starting in 1939, Theis[96] and his collaborators have extensively investigated the effect on the heat stability of chrome leather of various ions employed in pickling or in tanning. After tanning, the leather was split into grain, middle and flesh "layers"; these were then analyzed and

their behavior toward heat determined. Table 219 shows the result of pickling calf skin with sodium chloride and hydrochloric acid and then tanning it with chrome chloride of the basicities noted. Table 220 illustrates the effect of pickling with sodium sulfate and sulfuric acid and tanning with chrome chloride.

Table 219. Showing Cr_2O_3 Take-up during Tanning with $CrCl_3$ in the Presence of Chlorides Only.

Basicity $CrCl_3$	Cr_2O_3 in				Shrinkage Temp. (° C)			pH of Residual Chrome Liquor
	Grain G	Middle M	G-M	Total	G	M	F	
0	5.00	3.42	1.58	3.80	86.0	86.5	84.5	1.8
33	5.08	3.50	1.58	3.92	84.0	87.0	85.5	1.8
50	6.92	4.14	1.78	4.65	88.0	90.5	90.0	2.0
66	7.32	3.26	4.06	4.15	90.5	92.5	91.0	2.3

Table 220. Showing Cr_2O_3 Take-up during Tanning with $CrCl_3$ in the Presence of Neutral Sulfates.

Basicity $CrCl_3$	Cr_2O_3 in				Shrinkage Tem. (° C)			pH of Residual Chrome Liquor
	Grain G	Middle M	G-M	Total	G	M	F	
0	2.98	2.90	0.08	2.74	93.0	94.0	85.5	1.7
33	2.93	3.08	−0.15	2 74	90.5	93.5	90.0	1.7
50	4.02	3.18	0.84	3.69	94.0	97.5	97.5	1.9
66	5.62	1.51	4.11	4.06	100.0	100.0	99 5	2.2

Table 221. Showing Effect of Increasing Amounts of Sulfate Ion Present during Tannage with Basic Chromium Chlorides.

Moles Na_2SO_4 per Mole Cr	% Cr_2O_3 in				Shrinkage Temp (° C)		
	Grain G	Middle M	G-M	Total	G	M	F
0	4.95	3.12	1.83	3.44			
1/8	4.61	3.10	1.51	3.88	89	85	86
1/4	4.25	2.76	1.49	3.36	80	77	80
1/2	4.62	3.38	1.24	3.99	100	87	97
1/1	5 05	4.01	1.04	4.71	100	99	98
2/1	3.20	3.06	.14	3.09	94	96	96
4/1	2.95	2.65	.30	2.94	94	97	94

Table 221 gives the result of pickling the skin with 1.0 per cent sulfuric acid containing increasing amounts of sodium sulfate, so calculated as to represent the concentrations of Na_2SO_4 per mole of Cr shown in the table.

The three tables show that the presence of sufficient sulfate ion raises the shrinkage temperature of leather tanned with basic chrome chloride. That

is, when 0.50 mole Na_2SO_4 is reached but when 1.00 mole is exceeded the shrinkage temperature, as well as the fixed chrome, drops. Similar studies were then made employing basic chrome sulfate as the tanning agent, to which was added increments of various sodium salts—formate, oxalate, acetate and lactate. These salt additions varied from 0.25 to 3.00 moles per gram atom of Cr. Calf skin was pickled to various pH values with a mixture of sodium sulfate and sulfuric acid. The different chrome liquors noted above were then added to the exhaust pickle liquors and skin and tanning was performed for 24 hours, with constant agitation at room temperature. The experimental results may be summarized as follows. Addition of formate markedly increased shrinkage temperatures; small additions of oxalate increased shrinkage temperature but greater additions decreased it; acetate decreased heat stability in the case of all concentrations, but lactate had little effect.

In 1940, Holland[34] determined the shrinkage temperature of various calf leathers heated in glycerin. The bated and pickled skin was tanned with 33.0 per cent basic chrome sulfate to which was added various sodium salts, in concentrations varying from 0.25 to 0.50 moles per mole of $Cr(OH)SO_4$. The salts included: bicarbonate, formate, acetate, oxalate, succinate, adipate and phthalate. All these various added salts markedly increased the shrinkage temperature of the leather; they also greatly increased chrome fixations. There did not seem to be any direct relation between heightened shrinkage temperature and increased chrome fixation.

The important question of the thermolability of chrome leather will be further discussed when we consider the theory of chrome tanning. In view of both the practical and theoretical importance of the behavior of chrome leather to heat, it is to be hoped that our present knowledge of the subject will be extended and to include the influence of the protein-bound acid of chrome leather upon its heat stability.

Composition of Fixed Chrome Compounds

When we speak of the basicity of a chrome liquor, or of leather, we refer to the value calculated from the total chrome of the system and all its acid radicals. We do not necessarily mean that the chrome compound in the liquor or that fixed by the leather is of the basicity stated. Chrome liquors usually contain acid in two forms—free acid and acid combined with the chrome. When the amount of free acid is determined and is deducted from the total acid, the average basicity of the chrome in the liquor may be calculated. The term "average" is employed because the basicity value obtained is undoubtedly the mean of a number of different basicities. In the case of chrome leather, if we wash it completely free of acid radicals, we can say with certainty that its basicity is 100 per cent. But if such leather contains any fixed acid it is necessary to know how much of such acid is protein-bound

and how much is chrome-bound before the basicity of the fixed chrome compound can be calculated. This is the reason for the many investigations we have noted in considering the determination of the acid radicals in chrome leather. The actual basicity of fixed chrome has an important bearing on the theory of chrome tanning.

We have noted in Chapter 14 that, generally speaking, only those compounds which are basic, or which become so when dissolved in water, possess tanning power. In view of this, it will be well before proceeding to differentiate between what is experimentally known about the basic chrome compounds and what is derived by analogy.

There is much evidence to justify our belief that many basic chrome compounds exist and function in chrome tanning, as Stiasny has discussed at length in his book. But as such compounds are very difficult to isolate, we arrive at their composition mainly by speculation and analogy. A few basic compounds have been isolated, as follows. Small amounts of $Cr(OH)Cl_2$ and $Cr(OH)_2Cl$ were obtained by Bjerrum.[9] Richards and Bonnet [67] isolated a basic sulfate which they believed to be $Cr(OH)SO_4$. Stiasny[76] refers to the isolation of a crystalline hydroxo-disulfato-potassium-chromiate, and Theis has prepared the compounds described on page 493. Werner[105] isolated the $66\frac{2}{3}$ per cent basic chrome sulfate, $Cr_2(OH)_4SO_4$, which is insoluble in water but may be dissolved in acid solutions. As far as we know, no basic chrome chloride or sulfate compounds in which olation has been proved have been isolated.

Another view regarding the "basic chrome compounds" has been expressed by Weiser.[104] He suggests that the so-called basic compounds are really composed of hydrous chromic oxide which is peptized by adsorbed chrome sulfate or chrome chloride, as the case may be. Whatever the ultimate interpretation may prove, the conception of the basic chrome compounds is most useful for our purpose and we shall therefore employ it.

Throughout the literature many values are given of the basicity and composition of the chrome compounds fixed in chrome tanning, most of which were derived by analyzing leather which had been washed with water before analysis. As has been shown (pages 445 to 447), such washing vitiates the value of such results, and hence consideration of them can only confuse the issue. We cannot, for example, say that a leather contains fixed chrome of a certain basicity when we do not know to what extent we have removed its chrome-bound acid by the hydrolyzing action of water. For these reasons we shall consider in this section only those investigations which are most free from such objections.

As we have noted, when Gustavson in 1926 removed the protein-bound acid from basic chrome sulfate-tanned leather by his neutralization method, he found the fixed chrome of the two leather specimens to be 66.20 and 67.20

per cent basic, respectively. In 1929, Merrill and Niedercorn[58] repeate Gustavson's studies in an extensive series of investigations with chrom sulfate-tanned leathers and also with tanned hide powder. These author summarized their experiments as follows: "It will be noted that the leathe as tanned contained a complex of 67.7 per cent basicity. Since, as pointe out by Merrill, Niedercorn and Quarck, the diffusion-neutralization metho for chrome-bound sulfates always gives results that are slightly low, thi indicates that the chromium complex fixed by the protein was exactly two thirds basic, containing 0.5 sulfato group per atom of chromium. Treatin the leather with acid did not appreciably increase the acidity of this comple Partially neutralizing the leather with alkali decreased the total acid sulfat in direct proportion to the amount of alkali added, until the basicity of th complex had fallen to about half its initial value. Over this range the chro mium-bound and protein-bound acid both decreased linearly with increasin neutralization of the leather. When the chromium-bound acid had bee reduced to an amount corresponding to a complex of 82.6 per cent basicit [five-sixths basic complex (?)] the leather no longer contained protein-boun sulfate. Further neutralization merely reduced the acidity of the chromi complex. Since this paper was written, Gustavson has kindly called ou attention to the fact that our findings agree very nicely with some he obtaine in 1924. His work, too, indicates that the complex fixed initially is a two thirds basic sulfato-chromi compound, and that this complex is altered o washing to a more stable complex of approximately one-half the initia acidity."

In 1936, Merry[59] stated that the British Leather Manufacturers' Researc Association, in a private report sent to its members in 1922, suggested as working hypothesis that the compound fixed in basic chrome sulfate tannin was two-thirds basic.

In 1937, Cameron, McLaughlin and Adams[12] published experiments whic proved, by means other than those employed by Gustavson and by Merril and Niedercorn, that the chrome compound fixed in basic sulfate tannin is actually the two-thirds basic compound. They showed this to be tru regardless of the basicity of the original tanning solution employed. Thes authors tanned skin squares to equilibrium with increasing concentrations o basic chrome sulfate solutions of basicities which varied from 0.4 to 46. per cent.

They reasoned as follows: (1) If the chrome which hide substance fixe in sulfate tanning is always of the same basicity—regardless of the basicit of the chrome liquor, or the concentration of the liquor—a method of indirectl proving the basicity of such fixed chrome was available. (2) This would b possible from the experimental fact that when leather is tanned to tru equilibrium it shows the same basicity as the overall basicity of the tannin

system; and it may be reasonably assumed that there exists a direct quantitative relationship between amount of fixed chrome, amount of protein-bound acid, and amount of total available acid in the chrome liquor. (3) If sulfate tanning consists of the deposition of an insoluble chrome compound in and on the skin fiber, such deposition must result (as suggested by Stiasny in 1908) from the abstraction of acid from the solution by the skin fiber. The inherently insoluble chrome compound is held in solution by the acid. When sufficient acid has been removed by combination with the skin, the chrome becomes insoluble and is precipitated or deposited throughout the fiber, forming leather. (4) The only basic chrome sulfate which has been definitely isolated is the two-thirds basic salt; this salt is insoluble in water but is soluble in sulfuric acid solution. It has been shown by Gustavson and by Merrill and Niedercorn that such a compound is very probably the active tanning agent, as we have just noted. (5) When hide substance is treated with increasing concentrations of sulfuric acid and the values of fixed and free acid are plotted logarithmically a straight line results; see Table 222 and Figure 25, which illustrate the values obtained by McLaughlin and Adams.

Table 222

Grams H_2SO_4 Given per 100 Grams Collagen	Grams Acid Fixed	Grams Acid Unfixed	pH of Residual Acid Solution
4.00	3.72	0.28	2 35
5.00	4.44	0.56	2.05
6.00	4.72	1.28	1.75
6.40	5.14	1.26	1.75
7.20	5.24	1.96	1.60
8 00	5 38	2.62	1.50
8.80	5.82	2.98	1.40
9.60	5.92	3.68	1.30
10.40	5.93	4.47	1.25
11.20	6.06	5.14	1.20
12.00	6.08	5.92	1.15
13.00	6.37	6.63	1.10
14.00	6.44	7 56	1.08
15.00	6.46	8.54	1.00
16.00	6.65	9.35	0.98
17.00	6.66	10.34	0.92
18.00	6.83	11.17	0.90
19.00	7.00	12.00	0.85
20.00	8.78	11.22	0.90
21.00	8.88	12.12	0.83
22.00	9.50	12.50	0.80
23.00	9.72	13.28	0.79
25.00	9.50	15.50	0.73

With these thoughts in mind, Cameron, McLaughlin and Adams calculated the values shown in Table 223. The table includes values for 69 leathers tanned with increasing concentrations of basic chrome sulfate and of basicities varying from 0.4 to 46.0 per cent. It was assumed that the chrome compound fixed was the two-thirds basic throughout. It was further assumed that the

available acid sulfate in the liquor (f) should be derived by deducting from the total amount present (d) the amount required to make the total chrome present two-thirds basic (e), since this latter acid sulfate would not be available for reaction with the hide substance. In this way it was possible to calculate the values of protein-bound acid sulfate (i) and of the unfixed acid sulfate (j). When these two latter values were plotted logarithmically, it was found that

Table 223

(a) Grams Cr_2O_3 Given
(b) Grams Cr_2O_3 Fixed
(c) Grams Cr_2O_3 Unfixed
(d) Total Grams Acid SO_4 present
(e) Grams Acid SO_4 to make (a) 66⅔%
(f) Grams available acid SO_4 (d)-(e)
(g) Acid SO_4 to make (b) same basicity a original liquor
(h) Acid SO_4 to make (b) 66⅔%
(i) Protein bound acid SO_4 (g)-(h)
(j) Unfixed acid SO_4 in system (f)-(i)

0.4% Basic

(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)	(j)
6.00	4.01	1.99	11.31	3.78	7.53	7.56	2.53	5.03	2.50
9.00	4.61	4.39	16.96	5.67	11.29	8.70	2.91	5.79	5.50
12.00	5.12	6.88	22.61	7.56	15.05	9.66	3.23	6.43	8.62
16.00	5.44	10.76	30.16	10 09	20.07	10.26	3.43	6.83	13.24
20.00	5.84	14.16	37.70	12.60	25.10	11.01	3.68	7.33	17.77
24.00	6.33	17.67	45.25	15.12	30.13	11.94	3.99	7.95	22.18

2.8% Basic

(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)	(j)
4.22	3.82	0.40	7.77	2.66	5.11	7.04	2.41	4.63	0.48
6.32	4.52	1.80	11.64	3.99	7.65	8.32	2.85	5.47	2.18
8.45	4.87	3.58	15.55	5.33	10.22	8.96	3.07	5.89	4.33
10.55	5.12	5.43	19.41	6.65	12.76	9.42	3.23	6.19	6.57
12.63	5.42	7.21	23.23	7.96	15.27	9.97	3.42	6.55	8.72
14.77	5.62	9.15	27.20	9.32	17.88	10.35	3.54	6.81	11.07
16.85	5.66	11.19	31.00	10.63	20.37	10.41	3.57	6.84	13.53
18.95	5.75	13.20	34.90	11.95	22.95	10 59	3.63	6.96	15.99
21.10	5.82	15.28	38.80	13.30	25.50	10.71	3.67	7.04	18.46

15.8% Basic

(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)	(j)
8.00	5.72	2.28	12.75	5.04	7.71	9.12	3.61	5.51	2.20
11.00	6.48	4.52	17.55	6.94	10.61	10.33	4.09	6.24	4 37
14.00	7.22	6.78	22.31	8.83	13.48	11.51	4.55	6.96	6.52
18.00	7.82	10.18	28.70	11.35	17.35	12.46	4.93	7.53	9.82
21.00	8.14	12.86	33.50	13.25	20.25	12.97	5.13	7.84	12.41
24.00	8.58	15.42	38.30	15.14	23.16	13.68	5.41	8.27	14.89

17.5% Basic

(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)	(j)
6.36	5.21	1.15	9.94	4.01	5.93	8.14	3.29	4.85	1.08
8.45	6.00	2.45	13.20	5.33	7.87	9.37	3.78	5.59	2.28
10.58	6.49	4.09	16.54	6.67	9.87	10.14	4.09	6.05	3.82
12.67	6.94	5.73	19.80	7.99	11.81	10.85	4.38	6.47	5.34
14.81	7.23	7.58	23.14	9.34	13.80	11.30	4.56	6.74	7.06
16.90	7.40	9.50	26.40	10.65	15.75	11.56	4.67	6.89	8.86
19.04	7.76	11.28	29.72	12.00	17.72	12.13	4.89	7.24	10.48
21.12	7.90	13.22	33.00	13.32	19.68	12.35	4.98	7.37	12.31
23.28	8.22	15.06	36.37	14.67	21.70	12.85	5.18	7.67	14.03

Table 223—(*Continued*)

33.2% Basic

(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)	(j)
8.16	7.08	1.08	10.31	5.14	5.17	8.96	4.47	4.49	0.68
9.18	8.03	1.15	11.60	5.78	5.82	10.15	5.06	5.09	0.73
10.20	8.55	1.65	12.90	6.43	6.47	10.81	5.40	5.41	1.06
11.20	8.96	2.24	14.16	7.06	7.10	11.33	5.65	5.68	1.42
12.25	9.34	2.91	15.50	7.72	7.78	11.80	5.89	5.91	1.87
13.25	9.77	3.48	16.75	8.35	8.40	12.35	6.16	6.19	2.21
14.27	10.19	4.08	18.05	9.00	9.05	12.88	6.42	6.46	2.59
16.32	10.67	5.65	20.63	10.29	10.34	13.49	6.73	6.76	3.58
18.35	10.90	7.45	23.20	11.57	11.63	13.78	6.88	6.90	4.73
20.40	11.28	9.12	25.80	12.86	12.94	14.25	7.11	7.14	5.80
22.45	11.88	10.57	28.40	14.15	14.25	15.02	7.49	7.53	6.72
24.50	12.12	12.38	31.00	15.45	15.55	15.32	7.64	7.68	7.87

36.7% Basic

(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)	(j)
9.30	7.98	1.32	11.35	5.86	5.49	9.56	5.03	4.53	0.96
10.36	8.58	1.78	12.41	6.53	5.88	10.28	5.41	4.87	1.01
11.39	9.22	2.17	13.65	7.18	6.47	11.05	5.81	5.24	1.23
12.41	9.64	2.77	14.87	7.82	7.05	11.55	6.08	5.47	1.58
13.44	9.83	3.61	16.10	8.47	7.63	11.78	6.20	5.58	2.05
14.50	10.16	4.34	17.37	9.14	8.23	12.18	6.41	5.77	2.46
16.55	10.58	5.97	19.84	10.43	9.41	12.68	6.67	6.01	3.40
18.65	10.98	7.67	22.34	11.75	10.59	13.16	6.93	6.23	4.39
20.70	11.32	9.38	24.80	13.05	11.75	13.56	7.14	6.42	5.33
22.80	11.61	11.19	27.31	14.37	12.94	13.91	7.32	6.59	6.35

43% Basic

(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)	(j)
12.00	10.35	1.65	12.95	7.57	5.38	11.16	6.52	4.64	0.74
14.00	11.25	2.75	15.11	8.83	6.28	12.14	7.09	5.05	1.23
16.00	12.17	3.83	17.27	10.10	7.17	13.13	7.67	5.46	1.71
18.00	12.87	5.13	19.44	11.35	8.09	13.88	8.12	5.76	2.33
20.00	12.98	7.02	21.60	12.61	8.99	14.00	8.19	5.81	3.18
24.00	13.95	10.05	25.90	15.14	10.76	15.15	8.79	6.36	4.40

46% Basic

(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)	(j)
9.30	8.75	0.55	9.51	5.86	3.65	8.94	5.52	3.42	0.23
10.33	9.61	0.72	10.56	6.51	4.05	9.82	6.06	3.76	0.29
11.35	9.76	1.59	11.60	7.16	4.44	9.98	6.16	3.82	0.62
12.37	10.75	1.62	12.65	7.80	4.85	10.99	6.78	4.21	0.64
13.35	11.50	1.85	13.65	8.42	5.23	11.75	7.25	4.50	0.73
14.47	11.75	2.72	14.80	9.13	5.67	12.02	7.41	4.61	1.06
16.51	12.45	4.06	16.89	10.41	6.48	12.73	7.85	4.88	1.60
18.56	13.29	5.27	18.99	11.70	7.29	13.59	8.38	5.21	2.08
20.66	13.65	7.01	21.11	13.03	8.08	13.95	8.61	5.34	2.74
22.70	13.86	9.23	23.21	14.31	8.90	14.17	8.74	5.43	3.47
24.76	13.99	10.77	25.31	15.61	9.70	14.30	8.82	5.48	4.22

they all fell upon a single straight line, as shown in Figure 160. If any basicity value other than the two-thirds basic was employed in the calculations, the logarithmically plotted acid sulfate values produced a family of widely separated parallel straight lines, the position of each line varying with the original basicity of each liquor. It was only when the two-thirds basic

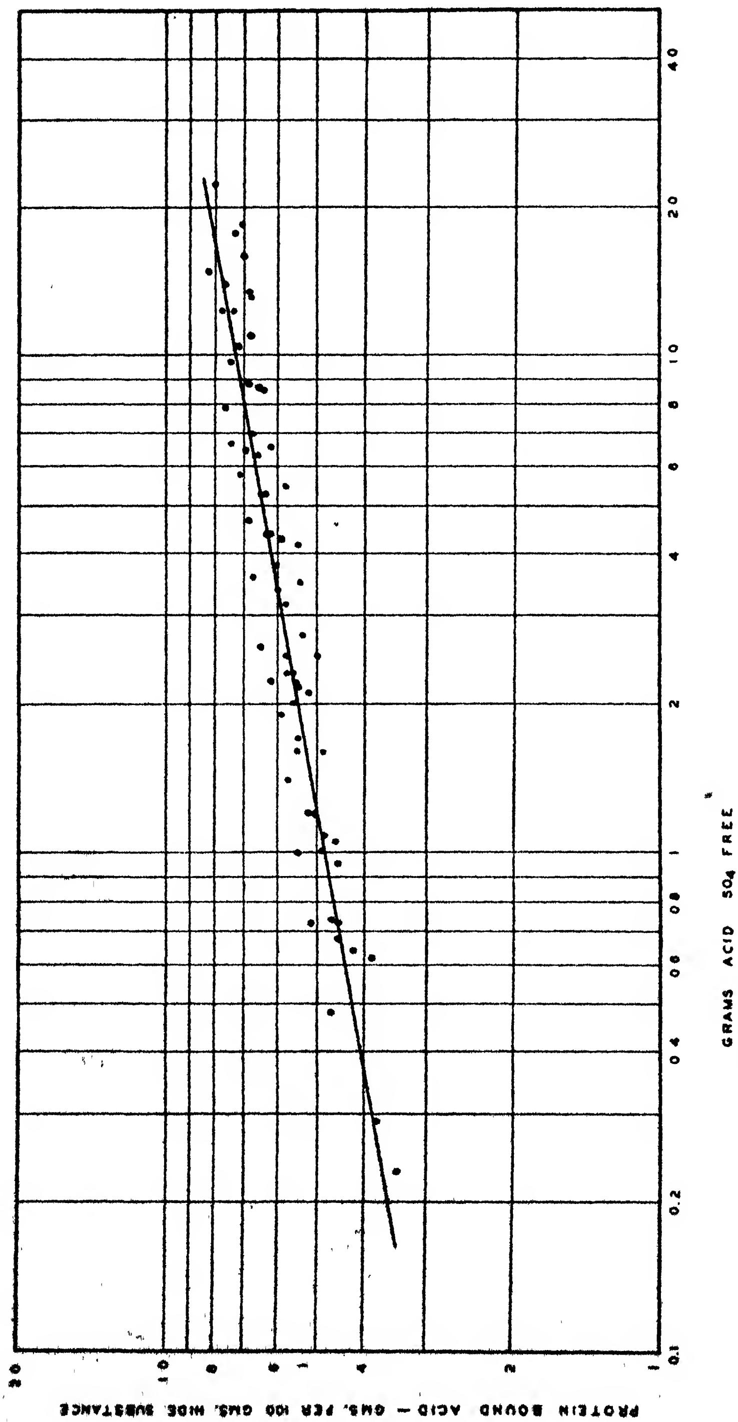


Figure 160. Distribution of Acid SO_4 between tanned protein and spent liquor, after tannage with chrome sulfate liquors of various basicities.

compound was assumed that all values fell upon a single line, regardless of liquor basicity. In view of this finding, these authors consider that there is no longer any doubt that chrome sulfate tanning consists of the deposition of the two-thirds basic compound and that hypothetical compounds of varying and unproved basicities are not involved.

In 1934, Küntzel, Riess, Papayannis and Vogel[42] tanned hide powder with basic chloride and basic sulfate solutions of 33.3 per cent and also with 50.0 per cent basicity. The powders were well washed with water after tanning; thus unfixed matter and an unknown amount of fixed acid were removed. The powders were then dried and aged for eight days before examination. Fixed chrome was then determined as well as the various acid radicals, employing the analytical methods described on pages 442 to 443. The results indicated the following basicities of the fixed chrome: from the 33.3 per cent basic chrome chloride 76.9 and from the 50.0 per cent 79.6; from the 33.3 per cent basic chrome sulfate 63.2 and from the 50.0 per cent 72.1. Practically all the chrome-bound acid was found to be complexly contained. From these experiments, the authors concluded that the chrome compound fixed in tanning is electrically neutral and that the combination between chrome and hide substance is not salt-like.

Molecular Size of Chrome Compounds

We noted in Chapter 14 that the molecular size of the chrome compounds employed in tanning may be increased by olation or polymerization and decreased by de-olation. Some workers, particularly Stiasny, believe the size of the compound to be of fundamental importance in the tanning process; Stiasny,[83] for example, states that compounds of too low molecular size will not tan, and that those that are too large are unable to diffuse adequately into the hide fiber. A number of attempts to measure the molecular dimensions of chrome compounds have been made and will now be described.

In 1923, Seymour-Jones[72A] prepared a 33.3 per cent basic chrome sulfate by reducing sodium bichromate with sulfur dioxide; it contained 269.9 grams Cr_2O_3 per liter. This highly concentrated solution was able to pass completely through hard filter papers which had been impregnated with 1.0 and with 5.0 per cent gelatin dispersions and which were subsequently hardened in 4.0 per cent formaldehyde solution. Similarly, complete diffusion was found when the concentrated solution noted (and when it was diluted 10 times) was placed in collodion bags and was dialyzed against cold water for 18 hours.

In 1927, Stiasny and Grimm[84] dialyzed various chrome solutions through parchment thimbles. It was thus found that 97 per cent of a zero basic chrome chloride (containing 1.0 per cent Cr) passed through a parchment thimble. This figure was reduced to 94 if the solution was boiled for 60 hours and was immediately cooled and dialyzed. The value for a 33.3 per cent

basic chrome chloride was 87, and this value was not altered by boiling and cooling, which indicated that olation changes did not greatly affect the liquor's dispersion. Sodium hydroxide was intermittently added to a zero basic chrome chloride until a basicity of 86 per cent was reached; this solution showed a slight Tyndall cone, and 98.0 to 99.5 per cent of the chrome compound it contained was retained by the parchment membrane. This experiment indicates a high degree of molecular complexity; but in none of the experiments just described (including those of Seymour-Jones) do we know the pore size of the filtering media.

In 1923, Jander and Scheele[38] measured the diffusion in water of chromium nitrates of varying basicities. Increasing amounts of sodium hydroxide were added as noted, and the diffusions were observed after the solutions had reached an equilibrium pH value, as indicated by the quinhydrone electrode. Average molecular weights were then calculated from the diffusion coefficients and are shown in Table 224.

Table 224

Basicity (%)	Moles NaOH added per mole Cr	Immediate pH value	Equilibrium pH value	Diffusion coefficient	Average molecular weight	No. Cr atoms per molecule
0.00	0.000	..		0.310	284	1.0
	0.235	3.53	2.73	0.290	325	1.1
	0.469	3.93	2.88	0.280	348	1.2
	0.703	4.24	2.99	0.280	348	1.2
	0.938	4.72	3.10	0.260	404	1.4
33.30	1.000*	...	.	0.250*	440	1.6
	1.170	4.76	3.18	0.230	516	2.5
	1.410	4.79	3.32	0.230	516	2.5
	1.640	5.03	3.40	0.160	1090	5.4
	1.880	5.23	3.51	0.150	1210	6.0
66.70	2.000*	.	..	0.125*	1750	9.0–13.0
	2.110	5.24	3.67	0.090	3370	26.0
	2.350	5.34	4.10	0.018	84000	650.0

* Interpolated

Reiss and Barth[71] have also employed diffusion methods in molecular weight determinations, as shown in Table 225, where all the solutions examined contained 0.7 per cent chromium.

Table 225

Basicity (%)	Molecular Weights			No. Cr atoms per molecule		
	Nitrate	Chloride	Sulfate	Nitrate	Chloride	Sulfate
0.00	346	267	608	1.0	1.0	2.0
33.30	442	484	796	1.7	2.3	3.7
50.00	758	737	947	3.4	4.0	5.2
54.00	...	...	1906	...	...	11.0
59.50	1190	1320	...	5.9	7.8	...
66.70	4080	1700	..	22.1	10.8	...

It will be noted that there is a wide divergence in the molecular weight value for the 66.7 per cent basic chrome nitrate, as shown in the two tables.

Riess and Barth found that heating either the 33.3 or the 66.7 per cent basic chrome chloride solutions employed had but little effect upon the molecular size determined, whereas heating the zero basic produced a slight increase. Addition of two moles sodium formate or sodium oxalate per mole Cr to the zero or 33.3 per cent basic nitrate solution did not affect molecular size. Adding 0.50 mole sodium sulfite per mole Cr to a chrome alum solution caused an appreciable and immediate increase in molecular size, which

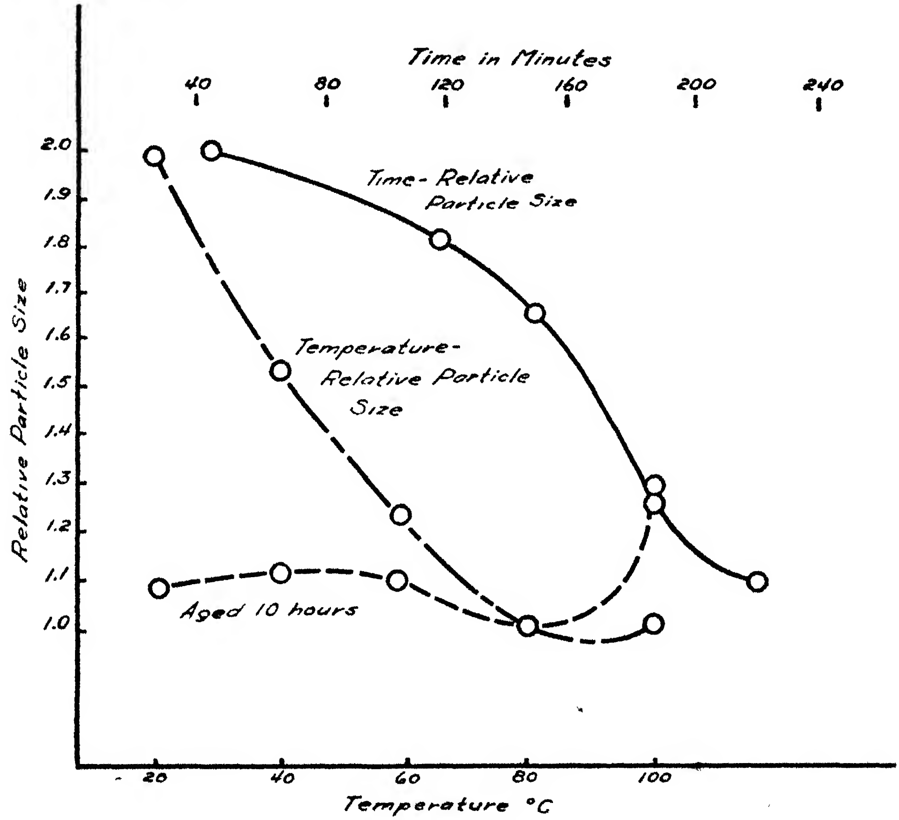


Figure 161

gradually diminished on standing. These authors point out that the chrome solutions most generally employed and whose basicity values range between 30 and 50 per cent contain from 2 to 5 chromium atoms per molecule, and thus fall within the crystalloidal range.

In 1937, Theis, Serfass and Weidner[97] studied the effect of time and of temperature upon the relative particle size of the chrome compounds contained in 1.0 per cent Cr_2O_3 solutions prepared from a 35.0 per cent basic dry chrome sulfate tanning extract. The values were determined by diffusion

methods which were similar to those employed by Riess and Barth. The term "particle size" designates the size of the chrome complex in its hydrated form. The results are shown in Figure 161, which indicates a rapid decrease in particle size as the liquor was heated, dropping from a value of 2.0 at 20° to 1.0 at 100°. When the liquors noted, which had been heated to the various

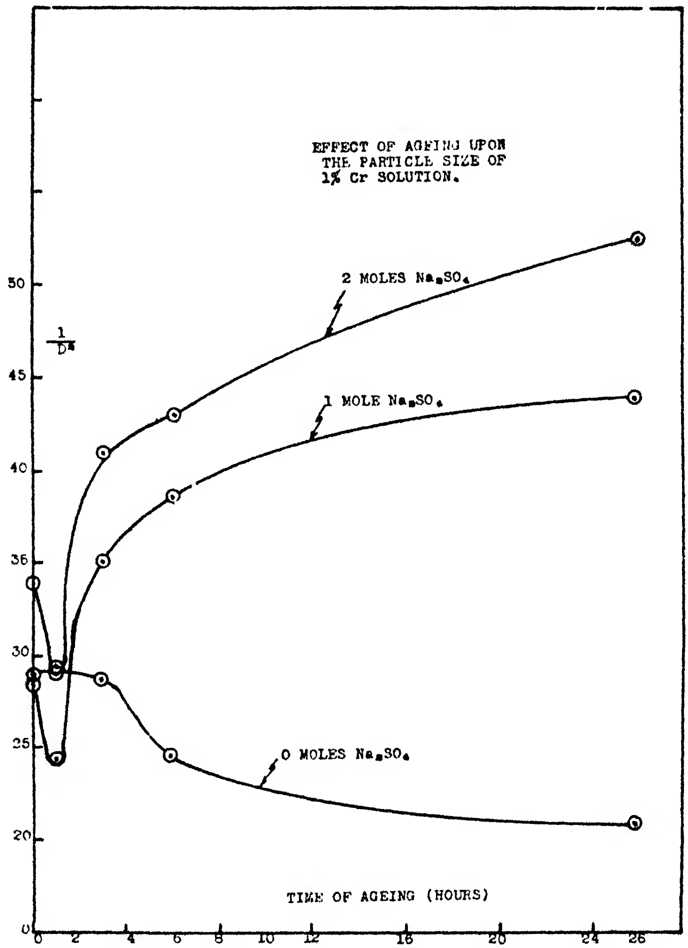


Figure 162

temperatures shown, were allowed to age for 10 hours, the particle size reached an approximately common level throughout. The uppermost curve of the figure illustrates the fact that in a freshly made solution at 20° the particle size decreases rapidly as a function of time, reaching an equilibrium value in 220 minutes.

Serfass and Theis[73] next studied particle size changes in an approximately pure basic 36.0 per cent basic chrome sulfate which contained no sodium sulfate. Solutions containing 1.0 per cent chromium were employed and the effects of aging and of the addition of sodium sulfate were studied. The various solutions were prepared by adding to the dry chrome compound the requisite number of moles of sodium sulfate per mole of Cr. To these dry mixtures was then added sufficient distilled water to produce 10.0 per cent chromium solutions, which were then boiled under reflux for 10 minutes. After cooling, the solutions were accurately diluted to 5.0 per cent Cr, were boiled under reflux for 30 minutes and were rapidly cooled; from them 1.0 per cent Cr dilutions were made. The results of particle-size studies, made by the diffusion method, are shown in Figure 162.

Figure 162 indicates a gradual diminution of particle size as a function of aging, although the change occurring between 5 and 26 hours is not marked. But great increases of particle size are induced by the presence of sodium sulfate. Serfass and Theis noted that the addition of sodium sulfate had progressively increased the pH values of the solutions, as reported by other workers, and that aging caused an initial drop in pH value, followed by a rise. They found also that the sulfate radicals which penetrated into the chrome complex during boiling were released upon aging. The amount of complexly bound sulfate in the aged 1.0 per cent solutions was independent of the total sulfate concentration of the solution; the value approximated one mole of sulfate for each two moles of Cr. The degree of olation was found to be independent of the amount of added sulfate. Their diffusion experiments indicated that the number of chromium atoms present in a single nuclear aggregate varied between 12 and 40, depending upon the conditions of preparation.

We cannot be sure from consideration of the various studies described in this section that the size of the chrome complex is of great importance, especially under the conditions of actual tanning, where great chrome concentrations or excessively high basicities are usually not employed. Some workers assume that the chrome of the solutions employed in tanning is in a highly dispersed state and that increases in its molecular size (leading to deposition and tanning) are induced by the removal by the skin substance of the acid which has dispersed it.

The Role of Acid in Cationic Chrome Tanning

Numerous workers have suggested that chrome fixation and acid fixation are mutually interdependent processes in cationic tanning. One school believes that the cationic chrome is fixed by acidic protein groups while the hydrolyzed acid of the solution is simultaneously fixed by the protein basic groups. Another school suggests that the fixation of acid by the basic protein

groups leads to the deposition or precipitation of an insoluble chrome compound throughout the skin fiber, whereby leather is formed, without necessarily involving the acidic protein groups. According to either line of reasoning, it follows that the amount of cationic chrome which may be fixed or deposited by hide substance is directly governed by the ability of the hide substance to fix acid. Thus, when the acid-combining capacity of the skin has been satisfied, no additional chrome fixation can occur; and, further, if the skin is kept acid-saturated during the tanning treatment it should fix no chrome whatever. Since these assumptions are of particular importance in our approach to the mechanism of cationic tanning, they will be discussed at some length.

The first attempt to determine the cationic chrome fixation capacity of acid-saturated hide substance was that of Küntzel and Riess,[46] in 1936. They reported that acid-saturated hide powder treated for two hours (the temperature of tanning was not stated nor was the method of tanning described) with 10 per cent Cr_2O_3 (on hide substance basis) of a zero basic chrome sulfate liquor which had been boiled 15 minutes fixed no chrome. In a similar experiment employing a 46 per cent basic liquor boiled 15 minutes, cooled, and then combined with sufficient sulfuric acid to render it zero basic, 0.22 per cent Cr_2O_3 was fixed. No description was given of the method employed for acid-saturation of the hide powder. It was stated that at the end of the two-hour tanning period the hide powder was shaken for two ten-minute periods with acid pickle solution. No description of the composition of the pickle solution was given. It would appear at first glance that the experiments of Küntzel and Riess indicated that no, or practically no, chrome was fixed under acid-saturated conditions. But their procedures were such, unfortunately, that no final conclusions can be drawn from their experiments. In the first place, chrome tanning proceeds at a very slow rate under highly acid conditions, and no appreciable fixation could be expected in a two-hour tanning period; in the second place, shaking the chromed powder with acid solution would tend to remove fixed chrome.

In 1937, Cameron, McLaughlin and Adams[12] demonstrated the quantitative relationship between acid fixation and cationic chrome fixation (see Table 223). Their conclusions as to the fundamental importance of acid fixation have been questioned by Gustavson,[30] who has stated, contrary to the findings of Küntzel and Riess, that acid-saturated hide substance does fix chrome. He visualizes two distinct types of reaction as occurring in cationic tanning: "A primary reaction governed by the number of free reactive acidic protein groups, and a secondary reaction independent of the state and nature of the groups but indicated to be localized to the peptide groups of the protein." And he states that if cationic tanning depends upon the fixation of hydrolyzed acid, it would not be possible for skin which has

been pickled to a low pH value to fix acid (and hence chrome) from a chrome liquor of a higher pH value. (In practical tanning skins may be pickled to a pH value of 2.5 or less and may then be placed in a chrome liquor of pH 3.0 and chrome will be fixed.) As proof of his two contentions just described, Gustavson performed the following experiments.

Pieces of bated, neutral pelt were presumably saturated with acid by pickling for 24 hours in a mixture of sodium sulfate and sulfuric acid to an equilibrium pH value of 1.0. The specimens thus pickled (as well as unpickled pieces) were then tanned in 37 per cent basic sucrose-reduced chrome sulfate which had previously been boiled and then made decinormal in H_2SO_4 and to which 3.0 per cent sodium sulfate had been added to prevent swelling of the pelt. The tanning period was 6 hours; the temperature of tanning was not stated, nor whether agitation was employed. The chrome concentrations given are shown in Table 226. It was not stated whether the leathers were washed or pressed after tanning to remove uncombined matters.

Table 226

No	Conc. of Tan Liquor gms Cr_2O_3 per l.	Per cent Cr_2O_3 fixed on H.S. basis: Unpickled Pelt	Pickled Pelt
1	14	4.2	1.0
2	23	4.5	2.1
3	47	4.6	3 2
4	93	4.8	4.3

In order to prove his second contention, as noted above, Gustavson tanned three skin specimens in the *same chrome solution*, under the following conditions. One piece was unpickled, the second contained 1.5 per cent H_2SO_4 and the third 4.5 per cent on a collagen basis; all three were placed in a basic chrome sulfate solution of the concentration and basicity shown in Table 227. Tanning time was 24 hours; the temperature of tanning was not stated, nor whether agitation was employed. From these experiments Gustavson concluded that in dilute chrome concentrations (such as would be employed in tanning) chrome fixation is regulated by acidity conditions, whereas the tanning mechanism in highly concentrated liquors is independent of acidity conditions.

Table 227

No	Nature of Tan	Per cent Cr_2O_3 fixed, on H S basis: Unpickled	Low Pickle	High Pickle
1	37% basic, 11 gms per l. Cr_2O_3	4.9	4.8	4.9
2	37% basic, 110 gms per l. Cr_2O_3	5.4	6.1	5.3
3	60% basic, 80 gms per l. Cr_2O_3	7 4	7.6	5.9

Gustavson regarded the fixations shown in Table 226 as proof that acid-saturated hide substance does fix chrome, the reaction being independent of acid conditions and occurring by means of a direct coordination between the protein peptide groups and the chromium atom. But it must be noted

that Gustavson was unfortunately not dealing with acid-saturated conditions; hide substance pickled to pH 1.0 is far from being acid-saturated (see Table 222). In order to prove definitely the point in question, it is necessary that the hide substance be actually acid-saturated, and that the chrome liquor contain sufficient free acid to insure that its free acid concentration is as great as the acid concentration of the pickle solution with which the hide substance had come into equilibrium. Otherwise, fixed acid will leave the pickled hide substance and flow into the surrounding chrome liquor. Thus, in order to maintain even the acid fixation reached at pH 1.0 in the pickle, Gustavson would have had to have added at least 8.5 grams H_2SO_4 per liter of chrome liquor, assuming that such added acid did not react with the boiled chrome liquor. In regard to the results shown in Table 227, if Gustavson had run his experiments to true tanning equilibrium he would have found exactly the same chrome fixations for each of the three specimens which were tanned in the same jar of liquor, as will be shown below.

In 1940, McLaughlin, Adams and Cameron[55] investigated the two contentions of Gustavson; their experiments will now be considered. In order to establish whether cationic chrome is fixed by hide substance under conditions which are actually acid-saturated, they proceeded as follows. Calf skin squares, as described on page 444, were pickled to complete acid-saturation by agitation for 24 hours at 70° F at 19 rpm in a solution containing 25 per cent sulfuric acid on hide substance basis and 32 per cent sodium sulfate; each 100 grams dry squares received 750 ml of pickle solution. At the end of the pickling period, 50 per cent basic chrome sulfate, sulfuric acid, and sodium sulfate were added and the various solutions were brought to the final dilutions shown in Table 228. It will be noted that sufficient acid was added in each case to maintain a uniform pH value throughout the entire series of solutions; this pH value was exactly the same as that of the exhaust pickle liquor. In this way, pickling equilibrium was maintained, so that no fixed acid left the skin. The concentration per ml of tan solution in sodium sulfate was maintained constant throughout; this prevented swelling or shrinking of the skin. The mixtures described were then agitated for 48 hours at 90° F at 19 rpm.

At the end of the tanning period, the squares were removed and drained, and were then pressed twice at 5000 pounds, after which they were promptly weighed and then dried, ground and analyzed. The amounts of chrome present in the hide substance for the various chrome concentrations are shown in the table. The question arises as to whether these amounts of chrome are actually fixed or deposited by the skin or whether they are merely present as a function of their solvent water being bound. If the latter is true, there should be a direct, quantitative relationship between the dilution of the liquor given and the amount of chrome present in the pressed skin.

But if the chrome is actually fixed or deposited no such relationship should be found. The percentage of "bound water" is shown in the table. These bound water values were obtained by adding together the weight of chrome, total sulfate, and hide substance and deducting their sum from the weight of the pressed specimen. It is at once apparent from the values of the table that there is a direct relation between the chrome present in the pressed skin and its bound water content. Taking, for example, the first experiment of the table: when 5 grams of Cr_2O_3 are dissolved in 1540 ml the concentration per ml is 0.00325 gram. The 100 grams of pressed skin contains 130.5 grams bound water; multiplying this by 0.00325 equals 0.42 gram Cr_2O_3, which is the theoretical amount to be expected in the pressed skin if no fixation or deposition has occurred. The value found by analysis is 0.37 gram. When the skin was treated with the same 5.00 grams Cr_2O_3, but in a dilution of 3100

Table 228

	Given 100 Gms Pickled H.S				Analysis after Tan--H.S Basis			
Ml of Solution	% Cr_2O_3	% Acid SO_4	% Na_2SO_4	Pressed Wt after Tan	% Cr_2O_3 Present	% Total SO_4	% Bound Water	Theoretical Cr_2O_3 in Bound Water
1540	5.00	40.62	32.00	240	0.37	9.14	130.5	0 42
3100	"	52.54	64.00	219	0.24	8.22	140.6	0.23
6280	"	85.34	128.00	255	0.13	8 07	146.8	0.12
1540	10.00	49.81	32.00	242	0.75	10.39	130.9	0.85
3100	"	61.65	64.00	247	0.48	8.69	137.8	0.45
6280	"	94.45	128.00	247	0.27	8.12	138.6	0.22
1540	20.00	68.36	32.00	230	1 21	10.34	118 5	1.54
3100	"	80.20	64.00	240	0.87	9.01	130.1	0.84
6280	"	113.00	128.00	244	0.54	8.36	135.1	0 43
1520	40.00	102.60	32.00	237	2.22	11.84	123.0	3.26
3100	"	117.20	64.00	237	1.56	10 17	125.2	1.61
6280	"	150 00	128.00	242	0.91	9 03	132.1	0.84
1500	80.00	173 00	32.00	212	3.97	15.35	92.7	4.91
3200	"	209.00	64.00	215	2.25	12.54	100.3	2 51
6480	"	289.40	128 00	237	1.73	11.61	123.7	1.52
1500	100.00	210.00	32.00	212	4.77	17 34	89.9	6.00
3150	"	237.00	64 00	225	2.94	13.25	108.9	3.45
6450	"	291.00	128.00	240	1.86	11.43	126.7	1.96

ml, the theoretical chrome value would be 0.23 and the actual is 0.24; when the dilution is 6280 ml the theoretical value is 0.12 and the actual 0.13. The same close agreement between theoretical and actual is noted throughout until 40.00 per cent Cr_2O_3 is reached. But even here, and also in the still higher concentrations given, it is seen that as long as the chrome concentration does not exceed 0.026 gram Cr_2O_3 per ml, the agreement between theoretical and actual values is excellent. When this concentration is exceeded the liquor is no longer able to adequately penetrate the acid-saturated skin, and the chrome present in such cases is less than the theoretical. It was found that the bulk of the chrome present in the pressed skin of Table 228 may be removed by washing with water, whereas chrome which is fixed or deposited can not be so removed. These authors therefore consider that no

chrome fixations whatever occur when an acid-saturation condition is actually maintained.

McLaughlin, Adams and Cameron next considered the results of Gustavson's experiments shown in Table 227, which, as noted, had not been run to tanning equilibrium, which fact accounted for the differences in chrome fixations found. These authors had pointed out that it makes no difference in cationic tanning whether acid is added to the tanning system by employing pickled skin or by adding the same amount of acid to the chrome liquor and using unpickled skin, that is, assuming that true tanning equilibrium is reached. If so, chrome fixation and leather basicity will be the same in each case. This is illustrated in Table 229. Twenty five-gram portions of

Table 229

Sample No.	% Cr_2O_3 Given	% Acid SO_4 Present	Overall Basicity of Tanning System	% Cr_2O_3 Fixed	% Total Acid SO_4 Fixed	Overall Basicity of Leather
Pickled Hide Substance						
1	13.20	14.65	41.4	9.41	10.51	41.0
2	"	16.10	35.5	8.56	10.53	35.0
3	"	17.56	29.6	7.80	10.60	28.2
4	"	19.04	23.8	7.10	10.34	23.1
5	"	20.50	18.0	6.12	9.60	17.2
6	"	21.22	15.0	5.78	9.49	13.3
Unpickled Hide Substance						
7	13.20	14.65	41.4	9.47	10.60	40.9
8	"	16.10	35.5	8.44	10.32	35.4
9	"	17.58	29.6	7.56	10.10	29.4
10	"	19.04	23.8	6.86	10.05	22.6
11	"	20.50	18.0	6.18	9.78	16.4
12	"	21.22	15.0	5.75	9.34	14.2

squares were pickled to equilibrium by continuously agitating for 48 hours at 70° F and 19 rpm in solutions containing 32 per cent Na_2SO_4 on hide substance basis and varying proportions of H_2SO_4: 1.55, 2.95, 4.43, 5.89, 7.35 and 8.07 per cent. At the end of the pickling period, 47.4 per cent basic sulfate chrome liquor (sulfur dioxide reduced) was added and the final volume of tan solution was made up to 250 ml. The six samples were then tanned for 48 hours at 90° F and with constant agitation at 19 rpm. They were then pressed twice at 5000 pounds and were dried and analyzed. At the same time a duplicate series was run with unpickled squares which were placed directly into the 250-ml tan solutions, in which had been incorporated the Na_2SO_4 and the varying amounts of H_2SO_4 employed in the pickled series. The twelve separate samples were all tanned at the same time.

We note from Table 229 that whether little or much acid is given hide substance, and whether in the pickle or in the chrome liquor, the net result as to chrome fixation and leather basicity is the same. And we also note

that the leather specimens—which have been tanned to equilibrium—all show essentially the overall basicity of the system in which they are tanned.

McLaughlin, Adams and Cameron next considered the last point raised by Gustavson, namely, that pickled skin could not fix acid from a chrome liquor of a higher pH value. This statement incorrectly infers that the pickled skin necessarily retains all its fixed acid during tanning. Table 230 illustrates what actually happens when pickled skin is placed in chrome liquor. Calf skin squares were pickled in a H_2SO_4/Na_2SO_4 solution and were then pressed twice at 5000 pounds. Analysis showed the pressed material to contain 8.40 per cent fixed acid SO_4 on hide substance. Five 10-gram samples were then placed in 100 ml each of 49.5 per cent basic chrome sulfate (sulfur-dioxide reduced) liquor, containing 15.05 per cent Cr_2O_3 on hide substance.

Table 230

Sample No.	Hours Tanned	% Fixed Cr_2O_3 H.S. Basis	% Acid SO_4 H.S. Basis	% Basicity of Leather	% Protein Bound Acid SO_4
1	0.5	1.81	6.85	−100.0	5.71
2	1.5	3.21	8.16	−34.0	6.13
3	12.0	6.86	11.30	+13.0	7.06
4	24.0	7.74	12.22	+16.6	7.33
5	48.0	8.52	12.92	+20.0	7.54

The overall basicity of the tanning system was thus 21.1 per cent. The five samples were then tanned at 90° F and with constant agitation for the time periods shown, and were then removed and were pressed twice at 5000 pounds and were dried, ground and analyzed. Protein-bound acid values were obtained by deducting from the total fixed acid SO_4 in the leather the amount required to make the fixed Cr_2O_3 $66\frac{2}{3}$ per cent basic.

Referring to Table 230, it will be recalled that the amount of fixed acid SO_4 of the pickled skin was 8.40 per cent. If we call this 100 and calculate the protein-bound acid of the leather as tanning proceeds in relation thereto, the following is found: After one-half hour of tanning the protein-bound acid value has dropped to 68; at $1\frac{1}{2}$ hours it is 73, at 12 hours 84, at 24 hours 87, and 90 at the end of the 48-hour tanning period. In other words, the pickled skin rapidly loses fixed acid at the start of tannage, forming a new system in the liquor of the drum. The skin then proceeds to come into equilibrium with the changed conditions, taking up acid and fixing chrome until final equilibrium is reached. At this point the leather shows practically the same basicity as the overall basicity of the tanning system.

In 1941, Theis[98] tanned pieces of bated goat skin with increasing concentrations of a $33\frac{1}{3}$ per cent basic chrome sulfate liquor the pH values of which were adjusted as shown in Table 231 and Figure 163. Tanning time was 24 hours, with constant agitation at 25°. At the end of tanning a specimen was tested for shrink temperature and a duplicate was pressed twice at 5000

pounds and was dried, ground, and analyzed for chrome. The pH values of the various chrome solutions were held constant throughout the tanning period. It will be noted that whereas appreciable amounts of Cr_2O_4 were found in the pressed leather at pH 1.0, the shrinkage temperature remained

Table 231. Effect of pH and Cr_2O_3 Concentration.

pH	Cr_2O_3 Conc Per Cent	Shrink Temperature °C No Salt	Salt*	Per Cent Cr_2O_3 in Leather No Salt	Salt*
1	1	47.5	43 0	1.15	0.57
	2	53.0	43.0	2.08	0.85
	3	47 5	45 0	3.14	1 36
	4	49 0	50 0	3.52	1.66
	5	57.0	53.0	3.96	1.95
2	1	69.0	72 0	3.41	2.09
	2	73.0	84 0	5.60	3.54
	3	85 0	90.0	6 82	4.07
	4	86.0	90 0	7 65	4.67
	5	96 0	92 0	7.90	4 87
3	1	115 0	109.0	6.81	5 26
	2	112.0	111.0	7 91	6 48
	3	112.0	111.0	9 15	6 98
	4	113 0	110 0	9 17	7.25
	5	107.0	107.0	9.28	7.29

* 1.0N NaCl used in the acid pickle.

essentially that of pickled, untanned skin. The temperature of tanning was then varied as shown in Table 232, from which it is seen that increasing the tanning temperature to 40° C (104° F) did not raise the shrinkage temperature sufficiently to indicate tannage. The tanning period at pH 1.0 was then increased to 120 hours at 20° with constant agitation. At the end of this

Table 232. Effect of Temperature.*

Tanning Temperature °C.	Shrink Temperature °C. pH of Tanning 1	3	Per Cent Cr_2O_3 Fixed pH of Tanning 1	3
20	41.0	101.0	0.48	4.55
30	45.0	110.0	0.73	6.37
40	50.0	115.0	1.25	7.64
50	..	119.0	...	8.08
70	. .	127.0	.	9.44

* Skin pickled for 6 hours in 1.0N NaCl solution containing H_2SO_4 to maintain either pH 1 or 3—then tanned in 1 per cent Cr_2O_3 solution for 24 hours at pH 1 or 3 at temperatures noted.

period the pressed leather contained 0.81 per cent Cr_2O_3 and showed a shrinkage temperature of only 44°.

Theis points out that if the degree of tannage can be measured by the shrinkage temperature of leather, his experiments indicate that no true tanning action occurs at pH value of 1.0. This statement holds true regard-

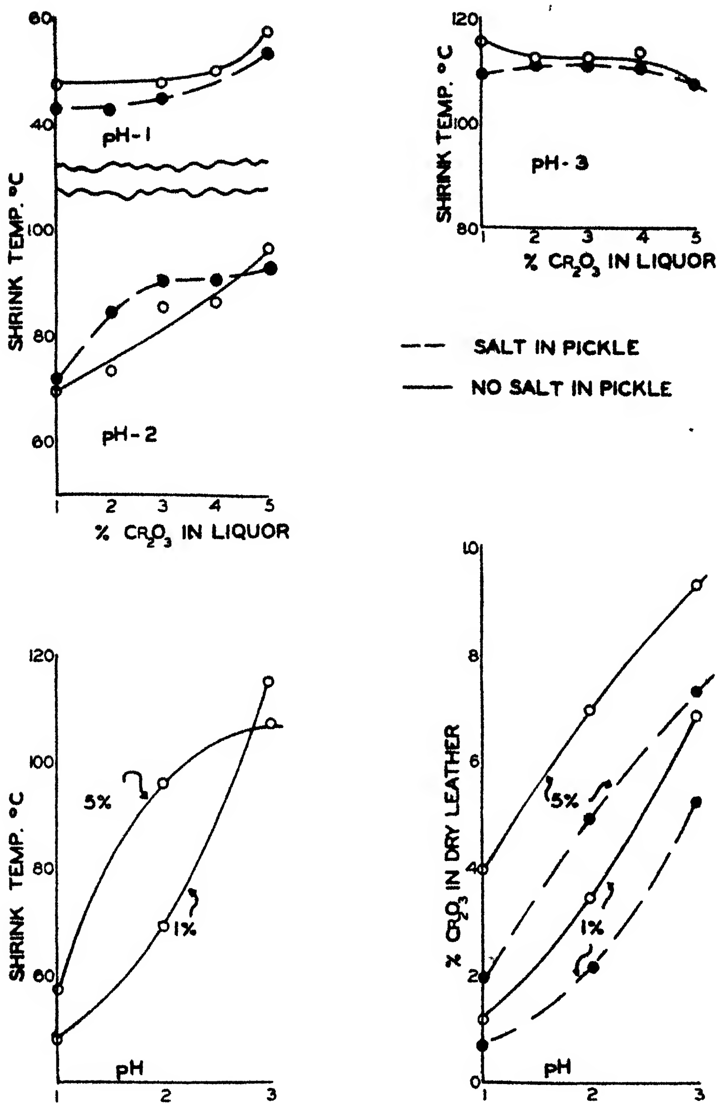


Figure 163. Showing the effect of concentration of chromic oxide in the chrome liquor upon shrinkage temperature of resulting leather tanned at different pH values.

less of type of pickle, chrome concentration given, temperature, or time of tanning.

In view of the findings of McLaughlin, Adams and Cameron and those of Theis, it may be definitely concluded that no cationic chrome tanning occurs under acid-saturated conditions.

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Chapter 16

Theory of Chrome Tanning

Having in mind the experimental studies and interpretations described in the preceding chapter, we shall now discuss the various theories which have been advanced to explain the mechanism of chrome tanning. If the reader will compare our present theoretical knowledge of this subject with the summary given in the second edition of this monograph, in 1929, gratifying progress will be noted. There are still many gaps in our knowledge of the theory of chrome tanning, there are diverging viewpoints, and it is improbable that any single theory will ever explain the many ramifications of the subject. But, even so, our fund of scientific knowledge has grown, and its application has resulted in improvements of manufacture and in the quality of chrome leathers.

The first theory of chrome tanning was that of the discoverer of the process, Friedrich Knapp,[14] in 1858. Knapp regarded chrome tanning as a covering of the outside surfaces of the hide fiber by an insoluble chrome compound produced by the hydrolysis of the chromium salt. This conception came to be known as the "physical" theory of tanning, in contradistinction to the "chemical" theory. This coating or physical theory proved untenable, however, when Küntzel,[16] and also Elod and Siegmund,[5] proved by microscopic means that the chrome in leather is not merely present on the fiber surface but is distributed throughout its interior as well.

In 1893, Fahrion[6] suggested that chrome leather was essentially a solid solution of tanning material in the hide fiber. And Körner,[15] in 1905, made a similar suggestion and believed the insoluble nature of the chrome compound explained the behavior of chrome leather toward hot water.

In 1908, Stiasny[29] suggested that the mechanism of one-bath tanning consisted of the fixation of hydrolyzed acid by hide substance and a simultaneous adsorption of the basic chrome component of the diffused tan liquor. It should be noted that this original conception of Stiasny is the basis upon which all later "deposition" theories have been built.

In 1917, Wilson[34] suggested that collagen, acting as an acid, and chromium hydroxide, acting as a base, combine to form the very stable salt, chromium collagenate. Wilson answered the obvious objection that both collagen and chrome are positively charged in cationic chrome tanning by postulating that

even though the charge on the collagen is predominantly positive, there still remain a small number of negatively charged groups scattered throughout its structure. Chromic ions diffuse into the fiber and combine with such groups. Further ionization of both collagen and chrome then occurs, and tanning proceeds to equilibrium. The hypothesis just described is now of historical interest only, but is mentioned because it is the first clearly stated "chemical" theory of chrome tanning.

In 1922, Thompson and Atkin[33] suggested the following tentative hypothesis. Since cationic chrome solutions may contain anionic complexes, they visualized that such negatively charged compounds combined with the positively charged collagen; and as such groups were thus removed, more would be formed. Seymour-Jones[27] demonstrated that this hypothesis could not be of general application, by tanning skin in a liquor containing no anionic complexes; this finding has since been repeatedly confirmed. But Thompson and Atkin's suggestion was of importance because it drew attention to anionic compounds and the necessity of explaining their combination with hide substance.

With this theoretical background before us, we shall now consider our present conceptions, dividing them into two general classes: (1) those in which specific groups in either or both of the two reactants are involved, and (2) those dealing more particularly with the deposition of chrome in and on the collagen fiber.

Class (1)

In 1921, Freudenberg[7] pointed out the pronounced tendency of the chromium atom to coordinatively saturate itself with nitrogen compounds, as in ammine-chromi salts, and with oxygen compounds, as in chromium compounds containing urea in their nuclei. He then suggested that this phenomenon might explain the mechanism of chrome tanning. According to this reasoning, such groups as $-NH_2$, $-NH-$ and $-CO-$ would penetrate into the chromium nucleus and displace other coordinatively held groups; but they would still remain a part of the collagen molecule, thus furnishing a connecting link between chromium atom and collagen. (Freudenberg's theory of vegetable tanning is discussed in Chapter 18.) Wilson[35] has speculated that the mechanism just described would make possible the combination of collagen with all the valency forces of the chromium atom, both primary and secondary. Thus, six negative protein groups could penetrate the chrome nucleus; the nucleus as a whole would then have three negative changes, and these charges could then further combine with three basic protein groups.

In a long series of papers, starting in 1924 and summarized in his book, Stiasny[30] and his collaborators have considered the mechanism of chrome

tanning from a different angle from that of his first conception, in 1908. In a lecture published in 1936, Stiasny[31] gives an interesting discussion of the mechanism of all types of tanning; from this lecture we quote his views on chrome tanning, as follows:

"Turning now to mineral tannage, attention must be drawn to the fact that only those chromium complexes are capable of tanning which contain hydroxyl groups. Chromium complexes which are free of hydroxyl groups and which do not by secondary changes (hydrolysis) form complexes containing hydroxyl groups, have no tanning capacity. Mineral tannage, therefore, can be explained in a manner analogous to vegetable tannage, the hydroxyl group of the tanning agent providing the secondary valencies, which attach the tanning agent to the hide. The analogy can be carried still further, the secondary valencies being in both cases attached to the hydrogen and not to the oxygen of the hydroxyl group. This is not only *a priori* most probable, considering the little affinity of oxygen (in the tannin molecule) to oxygen or nitrogen (in the collagen), but is also proved by the fact that chrome liquors with completely olated chromium complexes exert a strong tanning action.

"It must be remembered that the oxygen in such chromium complexes is coordinatively saturated (the coordination figure for oxygen being 3) and has no capacity for exerting secondary valencies. In the olated chromium complex, only the hydrogen of the hydroxyl group can provide the secondary valency necessary for the formation of a molecular compound with the hide collagen.

"The theory laid down for vegetable and for mineral tannages has to be supplemented by the claim of the considerable size of the tanning molecule. As far as chrome tannage is concerned, this claim is supported by the fact that the chromium salts bound in chrome leather are highly basic and, therefore, must contain large chromium complexes formed by olation. The two conditions, *viz.*, the presence of active hydroxyl groups and a considerable molecular size do not only apply for vegetable and mineral tannage but also for oil tannage.

"Those who prefer the idea that primary valencies are responsible for the combination with hide and tanning agent are localizing this action on the chemically active side chains in the polypeptides of which the collagen is built. Those in favor of secondary valencies causing combination between hide and tanning agent must be inclined to localize these secondary valencies on the peptide groups themselves, and it may be both the nitrogen and the oxygen of these groups which react with the tanning agent.

"According to our present knowledge, and especially to the view given above, the following definition seems justified: *Tanning means the transformation of the lyophilic groups in hide collagen into lyophobic groups.* This transformation can be obtained either by reactions between active groups of

the collagen and hydroxy groups of the tanning agent, due to secondary valencies on both components, the vegetable, mineral and fat tannages being examples of these kinds of action; or by such reactions between hide and tanning agent, whereby primary valencies are concerned, not necessarily including the process of salt formation. Examples of this kind of tanning are given by the formaldehyde tannage, quinone tannage and tannage by halogens."

Stiasny's views explain his belief that the nature of the charge on a chrome complex is of no great importance, since the coordinative power of its hydroxyl hydrogen is the governing factor. In connection with his contention that only those chrome compounds containing a hydroxyl group (or gaining same through hydrolysis) are capable of tanning, Küntzel, Riess and Königfeld[18] have offered the speculation that the chrome complexes fixed by hide substance are oxo rather than ol compounds. And the contention that the size of the chrome complex is of primary importance has been discussed on pages 523 to 527.

In 1924, Thomas and Seymour-Jones[32] tanned hide powder with various materials and then studied their behavior toward treatment with trypsin (see page 598). They found no tryptic digestion of chrome-tanned powder, and this finding has been essentially confirmed by Bergmann, Pojarlieff and Thiele,[1] who employed leather specimens. Thomas and Seymour-Jones postulated that since the enzyme is presumed to attack the polypeptide linkage, and since no digestion of the chrome-tanned powder occurred, it may be reasonably assumed that chrome may have combined with—and thus have inactivated—the peptide linkage. But we would point out that no final conclusions of this nature may be drawn until further experimentation has shown whether chrome itself inactivates the enzyme, and the pH value of the experimental system must be so controlled that enzyme inactivation will not occur as a function of the hydrolyzed acid of the leather. Merrill[24] has pointed out that we are not certain that the peptide linkage is the only point of attack by trypsin on collagen.

In 1926, Gustavson[8] stated his conception of chrome tanning as follows. "Fixation of cathodic chromium by hide substance, the regular type of one-bath chrome tanning, is regulated by the acidic and basic groups of collagen. Primary valence is probably concerned in the reaction with acidic groups and secondary valence with the basic groups of the proteins. The result is an internal complex salt. Anodic chrome fixation shows a maximum rate in the isoelectric zone of the proteins and is probably of residual valence type. This reaction is distinctly different from the combination of chromium cations with collagen." In 1927,[9] he explained the retardation of cationic chrome fixation by hide substance which has been previously deaminized or pretanned with formaldehyde, as follows. "The above data evidently favor the view

that the inhibition of cationic chrome fixation by hide protein possessing a less number of reactive basic groups than regular protein is connected with the diminished acid combining capacity of the structurally altered hide powder."

In 1931, in view of the enzymatic experiments of Thomas and Seymour-Jones described above, Gustavson[10] revised his views regarding cationic tannage and considered the mechanism to consist of a primary reaction between the complex chromium cation and the collagen carboxyl groups and, in addition, the formation of a molecular compound between chrome complex and collagen peptide group. In other words, he contemplated a ring structure in which the complex would be attached at two points of the protein molecule. This concept is not related to the bridging of adjacent polypeptide chains. Küntzel and Dröscher[20] have criticized Gustavson's conception, stating that peptide groups react with chrome in strongly alkaline solutions only and that reaction in acid solutions is quite unlikely. Merry[25] has stated that since cationic tanning occurs on the acid side of the isoelectric point of collagen, the amount of ionized carboxyl groups would be very small and that the electro-valent primary reaction postulated by Gustavson is extremely improbable. Gustavson[11] has answered this objection by pointing out that, according to Jordan Lloyd's[21] titration curve of collagen, 75 per cent of its carboxyl groups are in the ionized state at a pH value of 3.0, which is a normal pH value in chrome tanning.

Following the announcement by Spiers,[28] in 1934, of his "bridging" theory (to be described), Gustavson[12] revised his views in 1936 and 1937 as follows: "The resistance of chrome leather towards the action of boiling water is considered to be due to the presence of connecting links between the structural units in the chrome-collagen compound. The chrome complexes serve as bridges through participation of ionic-covalent forces to the acidic protein groups of one chain and of coordination valency to the discharged basic groups of another peptide chain." In 1939, in discussing the role of hydrolyzed acid in cationic tanning (see pages 527 to 535), Gustavson[13] further revised his views as follows. "It is demonstrated that even in dilute solutions of basic sulfates, besides the dominating reaction which is controlled by the activity of the acidic groups of the protein, another type of chrome fixation occurs. The latter process evidently is not influenced by the state of the acid-binding protein groups. For many reasons, only one will be mentioned further on in this connection; the primary reaction does not appear to be governed by the acid hydrolysis of the chromic salt. Instead, a great number of observations, to be given in detail in another place, support the view that the main fixation process rather should be formulated as a coupling of cationic chrome complexes with the COO^- groups of the hide protein. With increased concentration of the chromic sulfates, the pH independent attachment of

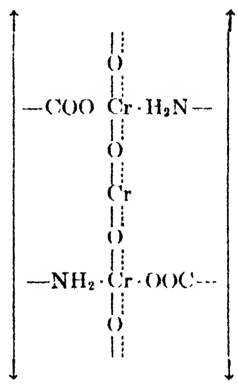
chromic compounds grows in importance, and in strong solutions it dominates. This latter process is indicated to be of the nature of a direct coordination of the protein, probably by means of its --CO—HN-- groups, to the chromium atom." It will be recalled in this connection that McLaughlin and Adams[23] have shown that no type of chrome fixation occurs, with either dilute or highly concentrated liquors, under conditions which are actually acid-saturated.

In a paper published early in 1934, Spiers[28] discussed the mechanism of tanning and made the following significant statement. "On tanning collagen the shrinkage temperature is raised by an amount depending upon the tanning agent used. In the case of chrome tannage the shrinkage temperature may be much over 100°. This alteration seems to be of profound importance from the point of view of the nature of the tanning process. Does the tanning agent prevent the shrinkage by a mere mechanical blockage, or does it penetrate the micelles and somehow rivet together the polypeptide chains, preventing them from undergoing spatial alterations? One would imagine that chrome compounds could be especially effective in linking together the chains because chromium atoms form very stable coordination compounds containing both carboxyl and amino groups. Thus a chromium atom could serve to bind together two adjacent chains, by a carboxyl group from one and an amino group from the other." The ideas here expressed by Spiers represent the first suggestion to be found in leather literature of the riveting together of adjacent protein chains by a tanning material. His theory has been accepted by numerous workers and has been variously applied by them.*

In 1934, Küntzel, Reiss, Papayannis and Vogel[17] stated that the chrome complex combined with collagen in cationic tanning was electrically neutral and that all its acid radicals were complexly held (see page 523). In view of this, they contended that the chrome compound could not be held by a salt-like linkage. But after the publication of Spiers' bridging theory, Küntzel and Riess,[19] in 1936, restated their conception as follows. They assumed that polynuclear chrome complexes (in which the Cr atoms are linked together by oxygen atoms) form bridges between adjacent polypeptide chains. The amino group of one chain and the carboxyl group of another are coordinated with a Cr atom, as shown on page 544.

They state the chrome complex must be large enough to span the space between the fibrils and that the carboxyl groups must be ionized, so that they can react with the peripheral Cr atoms of the complexes. They speculate that when chrome leather is treated with acid the acid combines with its amino groups, thus breaking the secondary valence bond between such groups and the chrome complex. But they assume that after such treatment there would be sufficient remaining primary valence bonds between Cr and carboxyl

*In other words, Spiers applied the original cross linkage postulation of K. H. Meye and H. Mark, *Aufbau der hochpolymeren organischen naturstoffe*, Liepzig, 1930.

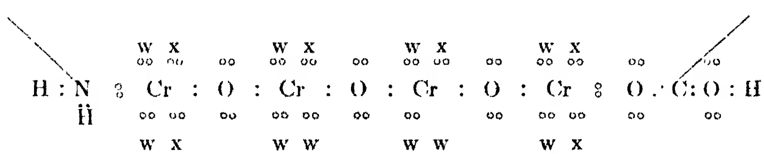


groups to prevent acid swelling of the leather. This latter linkage is broken only by an excess of added acid. They liken the changes which occur when chrome leather is dried to those presumed to take place in the evaporation of a cationic chrome extract; that is, ionically chrome-bound acid radicals enter the chrome complex and aquo groups are lost.

In 1935, Jordan Lloyd[22] discussed the mechanism of tanning. She first called attention to the influence and importance of the size of the tanning molecule (see page 621). She then summarized the tanning action as follows: "To turn skins into durable and useful leather, therefore, there must be an inactivation of all the active centers in the molecule (collagen), thus leading to loss of chemical activity, elimination of water and, as a direct consequence, a protection of the peptide links. This inactivation should as far as possible be an irreversible change. . . . . To sum up, tanning means suppression of the chemically active centers of the R groups (side chains) of the collagen molecule, with dehydration and protection of the peptide links of the molecular backbones. The carboxyl groups of the collagen, which form negatively charged centers in water, will be inactivated by the acid character of the tan liquors and the amino groups which form positively charged centers in water by chemical interaction with the tanning agent. The first stage of this, with vegetable tannins at least, and more than probably with chrome tanning salts also, is the formation of a salt in which the collagen acts as a base and the tanning agent as an acid. Later this electrovalent link is transformed into a covalent link. Supression of the positively and negatively charged centers will lead to loss of bound water by the collagen. With vegetable tanning, the hydroxyl groups of the tannin probably also interact with the imino groups of the polypeptide backbone. This will lead to protection of the backbone from proteolytic enzyme action. Whether a similar action occurs with chrome salts is not known."

In a series of papers starting in 1936, Wilson[36] considered the phenomena of tanning from the electronic standpoint. His views regarding chrome

tanning may be summarized as follows. The imino-carbonyl link is the weakest part of the collagen structure. Chrome tanning consists of the replacement of these weak links by stronger links of atomic chains. This occurs by the displacement of coordinately held H_2O (or other) molecules by amino or carboxyl groups, whereby long and stable coordinate links are formed (see formula below). The short imino-carbonyl links may also be replaced by the longer links. This conception is not unlike that of Freudenberg, described above. Wilson did not state whether the mechanism just described referred to the bridging of adjacent protein chains, or whether the chromium is attached to a single chain only.



Class 2

The first deposition theory (in contradistinction to Knapp's surface-coating theory) was announced, as we have noted, by Stiasny in 1908. Essentially similar views were expressed by Procter[26] in 1910, and by Burton[2] in 1922.

In 1932, Elöd and Siegmund[5] removed all electrolytes from cationic-tanned leather by means of electrodialysis. The leather so treated did not shrink in boiling water and showed an isoelectric point between 5.7 (the value found for the untanned collagen employed) and 7.3, the value for pure chromium hydroxide. In view of this isoelectric value, these authors concluded that the chromium hydroxide present in the dialyzed leather was in the free state—that it was not chemically combined with collagen. In support of this conclusion, they placed dehydrated skin in an alcoholic solution of chromium ethylate and then in warm water, whereby chromium hydroxide was formed and was deposited throughout the collagen fibers. This leather also resisted the action of boiling water. They then tanned skin to equilibrium with cationic chrome, after which they removed by electrodialysis its total acid radical content and then retanned it; these operations were repeated until 44.60 per cent Cr_2O_3 was fixed. Retannage in the absence of electrodialysis resulted in only minor increases of chrome fixation. This indicated that acid fixation and chrome deposition are mutually interdependent processes. When the acid-combining power of skin is exhausted, no further tannage occurs. Microscopic examination of all the various leathers described showed the chrome to be uniformly distributed throughout the collagen fibers. Elöd and Siegmund interpreted their experiments as follows. One-bath chrome tanning is a deposition process; the skin fixes (reversibly) the free acid of the liquor, which leads to further hydrolysis and to the deposition of

a highly basic salt. Both chromium hydroxide and other highly basic compounds can tan, providing they attain suitable particle size (through hydrolysis or olation) and are uniformly distributed throughout the fiber. In 1934, Elöd and Cantor[4] stated that the deposited chrome compounds react with active protein centers by means of secondary valencies.

In 1936, Merry[25] stated that the British Leather Manufacturers' Research Association, in a private report issued in 1922, had suggested as a working hypothesis that cationic chrome tanning consisted of the deposition of a 66⅔ per cent basic chrome sulfate in and on skin fibers. It was assumed that part of this basic salt combined chemically with collagen, possibly by an interaction involving its NH_2 groups and the OH groups of the chrome salt. In other words, a "deposited basic salt and a compound of this with collagen." In support of this latter statement, Merry found that if freshly tanned leather is treated with oxalic acid, about one-third of the total fixed Cr_2O_3 is not stripped unless the temperature of the treating solution is raised. He suggested that this one-third is chemically combined with collagen, whereas the other two-thirds is the deposited, unchanged basic salt. Cameron, McLaughlin and Adams[3] repeated this experiment, employing both wet, freshly tanned leather and leather that was dried and aged. They found in both cases that treatment with oxalic acid removed only part of the fixed or deposited basic sulfate, which seemed to confirm Merry's finding. But they noted that when sufficient chrome was stripped, the now partially tanned leather was greatly swollen by the acid, and this inhibited the outward diffusion of the reversed chrome. When the acid solution was replaced with a saturated solution of sodium oxalate the swelling was reduced and the remaining chrome in the skin diffused out at a rapid rate. Their finding has recently been confirmed by Lollar.

In 1937, Cameron, McLaughlin and Adams[3] published experimental evidence which proved that there is a quantitative relationship between acid fixation and subsequent chrome deposition in the case of chrome sulfate tanning. They also showed that the deposited chrome is always the 66⅔ per cent basic chrome salt, regardless of the original basicity of the chrome liquor used, and that such fixation or deposition of chrome is a completely reversible process (see pages 505 to 510). In 1940, McLaughlin and Adams[23] proved that when the acid-combining capacity of collagen is satisfied, the collagen has no ability to fix cationic chrome (see pages 530 to 534).

We stated at the beginning of this chapter that encouraging advances have been made in our knowledge of the theory of chrome tanning during the past fifteen years. This progress has gone hand in hand with a better understanding of the chemistry of proteins in general and of collagen in particular. There can be but little doubt that future progress in the theory and applica-

tion of chrome tanning will go hand in hand with the growth of protein chemistry. The bridging theory of Spiers is a conception which is both logical and attractive, and one of the most important problems of the future is the proof of its correctness. There would now seem to be no reasonable doubt that, in the case of cationic tanning, at least, the process is primarily concerned with the fixation of the acid of the chrome solution which has diffused into the skin fiber and the subsequent deposition of a highly basic insoluble chrome compound. But it remains to be proved whether this compound is merely deposited, or whether it chemically combines with the skin protein and, if so, in what manner. Much work remains before we can adequately explain anionic tanning.

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Chapter 17

Vegetable Tanning

When animal skin is placed in an aqueous infusion of certain woods, barks, nuts, roots or leaves, it undergoes a remarkable change. Whereas the moist original skin is easily decomposed by warm water or by bacterial enzymes and is quickly shrunk if subjected to heat, it now has entirely new characteristics: it no longer dissolves in water, is comparatively unaffected by bacterial enzymes, and is capable of resisting a great deal of heat. In other words, it is no longer raw skin but has become vegetable-tanned leather. This means that the vegetable "tannins" of the solution have in some manner reacted with the skin proteins and that a new compound is formed. It is the purpose of this chapter to describe and to discuss what is known of this reaction. The reader will recognize that the problem to be considered—entailing, as it does, the reaction between complex proteins and the complex organic bodies termed tannin constitutes a question which is at once the delight and the despair of the leather chemist. Vegetable tanning is of ancient origin, but even today we have much to learn of its fundamentals.

This is not due to laxness on the part of the leather chemist; it is because he has had to await the advances of modern protein chemistry and physics and newer methods in organic chemistry; and it is a source of satisfaction that leather chemistry has itself contributed in a substantial manner to these fields.

Before proceeding with the scientific discussion of vegetable tanning, it will be well to understand something of its economics and also something of the general methods of tanning.

The great bulk of all sole, harness, and belting leathers produced in the United States is vegetable-tanned; these are termed "heavy" leathers and are made from the heavy hides of mature animals, such as the steer and the cow. A smaller proportion of the country's vegetable tannin requirement is used in the production of various lighter leathers from the smaller skins of calf, sheep, goat, etc. A further small proportion is employed in the manufacture of the so-called "retan" leathers, that is, leathers made from medium- and heavy-weight hides, such as cow, which are first tanned with chromium compounds and then retanned with vegetable tannin. In order to accomplish all this vegetable tanning, the United States consumed some 100,000 tons of

100 per cent tannin in 1939. This tremendous quantity of tannin came from numerous botanical sources and from various parts of the world. It is interesting to note the percentage relationships of the various kinds of tannin to the total used, as shown in Table 233.

Table 233. Sources of Tannin.

	Percentage of Total
Quebracho wood	44.2
Mangrove bark (cutch)	5.4
Myrobalans	4.6
Wattle bark	3.8
Valonia	3.6
Gambier	1.0
Sumac	0 9
Chestnut wood	30.8
Spruce (by-product of paper mills)	3.0
Oak bark	1.7
Hemlock bark	1.0
	100.0

The first seven materials shown in the table are imported, and it will be noted that they constitute 63.5 per cent of the total. The quebracho tree (*Quebracho lorentzii*) grows in South America. Mangrove bark is obtained from several species of *Rhizophora* which grow in various tropical countries, the most important source hitherto being Borneo. Myrobalans are the dried nuts of *Terminialia chebula*, native to India. Wattle bark is derived from various species of *Acacia* grown in Australia and South Africa. Valonia comes from the cups and beards of the acorns of the Turkish oak, *Quercus aegilops*, indigenous to Asia Minor. Gambier is obtained from the leaves and twigs of *Nauclea Gambir* found in India and the East Indies. Sumac is secured from the leaves of *Rhus Coriaria* grown in Sicily. The remaining four materials of the table are of domestic origin. Chestnut wood, *Castanea dentata*, is found in a number of the eastern and southeastern states. Spruce extract is a by-product of the manufacture of paper from spruce wood. Oak bark comes from several species of oak, *Quercus robur*, *Quercus densiflora*, and *Quercus prinus*. Hemlock bark is derived from the hemlock tree, *Tsuga canadensis*.

The table shows, therefore, that the United States is in the unhappy position of importing two-thirds of its tannin supply. This is aggravated by the fact that the chestnut tree (our largest domestic tannin source) is being rapidly exterminated by a fungus called *Chestnut Blight*, for which no effective control has yet been found, and by the fact that our supplies of oak and hemlock barks have dwindled. Steps in two directions are being taken to meet this situation. Investigations are under way to find and utilize other domestic tanning materials, and important studies are now in progress in

several laboratories with the object of synthesizing effective organic substitute tanning agents.

Table 233 also gives us a picture of the evolution of domestic vegetable tanning methods. Fifty years ago the great bulk of American vegetable leather was tanned with chestnut oak and hemlock bark tannin, used separately or in admixture. Today the great bulk is tanned with quebracho, chestnut, and mangrove tannin. Coincident with this change of tanning material has been a continuous shortening of the time required for heavy-leather tanning. It was not unusual for the sole leather tanner of fifty years ago to tan his hides for 180 days; today this tanning period has been cut to 30 days in many instances.

Tanning Methods

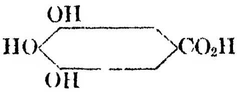
We shall not attempt in this book to give detailed descriptions of tannery operations, since lack of space prevents it.* But for our purpose it may be briefly stated that the limed, unhaired, delimed, and usually bated hide or skin is brought into contact with an aqueous infusion or solution of vegetable-tanning materials, usually a mixture of several. The first tan liquor to reach the untanned hide or skin must be both weak in concentration and mellow (lacking astringency) in character; otherwise, the skin's outer surfaces would be tanned too rapidly, imparting undesirable qualities to the leather, and preventing diffusion of the tan liquor into the skin's interior. As tanning proceeds, that is, as the reaction between tannin and skin collagen progresses, it is necessary to increase continuously the concentration and astringency of the tan liquor until the desired degree of tannin fixation has occurred. To regulate properly this seemingly simple procedure, long experience is required on the part of the tanner and a great deal of knowledge on the part of the leather chemist. This is because the course of the tanning reaction is influenced by many factors: time and temperature of reaction, the chemical and physical condition of the hide or skin and of the tan liquor, the acidity and the pH value of the liquor and its concentration, the presence of both organic and inorganic substances other than tannin (*i.e.*, substances known as "nontannins") in the liquor, and, finally, the degree of subdivision of the liquor constituents. All these factors and their influence must be understood before any intelligent approach can be made to an understanding of the real nature of the reaction. We shall therefore discuss them before taking up the theory of vegetable tanning.

Chemistry of Tannins, Nontannins, and Insolubles

Tannins. Chemical investigation of the constitution of the tannins may be said to have started some 160 years ago with the experiments of the

* An excellent description of modern tanning methods is given in J. A. Wilson's "Modern Practice in Leather Manufacture," Reinhold Publishing Corporation, New York. See also "Tanning Processes" by August C. Orthmann. In press.

Swedish apothecary Carl Wilhelm Scheele, one of the discoverers of oxygen. Scheele allowed an aqueous infusion of Turkish oak galls to be fermented by molds. He noticed that a crystalline substance settled below the mold layer, and he was able to recrystallize this substance from water. He called the substance "sal essentiale gallurum," and it became known in the literature as gallic acid, having the formula:

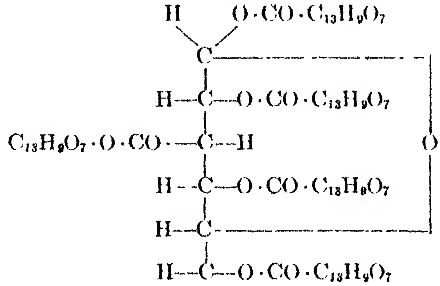


that is, a trihydroxybenzoic acid. Scheele thought the peculiar action of the mold probably consisted in the removal of impurities which prevented the crystallization of a *preformed* substance. It was soon pointed out, however, that crystalline gallic acid and the amorphous gallotannic acid were not identical. The Flemish botanist van Tieghem discovered that the mold *Aspergillus Niger* was especially active in fermenting gallotannin and showed that only a few milligrams of mold mycelium were able, under proper conditions, to decompose some 50 grams of gallotannic acid. Van Tieghem ascribed this action to a mold enzyme, *tannase*, and noted that an alcoholic fermentation set in simultaneously. Liebig had already mentioned such fermentation, which he attributed to the presence of sugar in the gallotannic acid molecule. Liebig and Pelouze had found in 1834 that when gallotannic acid was acted upon by dilute acids, the same gallic acid which had been obtained by fermentation was now split off by the acids.

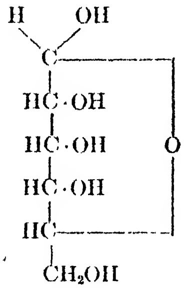
The literature of the remainder of the nineteenth century contains many investigations of the constitution of tannin, none of which are very conclusive. We now know that the foundation of our present knowledge of the chemistry of tannin was laid in the early work of Scheele, van Tieghem, Liebig, and their contemporaries. But it remained for Emil Fischer and his associates, Max Bergmann and Karl Freudenberg, to build the superstructure.

In 1912, Fischer and Freudenberg[34] hydrolyzed carefully purified gallotannic acid with 5 per cent sulfuric acid and obtained some 90 per cent of its weight of gallic acid and 7-8 per cent glucose. (The actual values of gallic acid and glucose were low, since control experiments with mixtures of them showed that losses of both compounds occurred during hydrolysis.) Since this yield of glucose was much too small for an ordinary glucoside of gallic or digallic acid, they considered that the combination must be of an ester nature, and they reasoned that a compound of one molecule of glucose and five molecules of digallic acid (that is, penta-*m*-digalloyl-glucose) would, upon hydrolysis, yield 100.0 per cent of its weight of gallic acid and 10.6 per cent of glucose. Six years later Fischer and Bergmann[33] succeeded in synthesizing such a compound and proved it to be an isomer of Chinese

nutgall tannin. The structural formula of the synthesized gallotannic is shown below, under I, while that of glucose, II, is included for comparison.



I.



II

The above tannin formula is probably typical of most of the "hydrolyzable" tannins about to be described.

It must not be assumed that this successful synthesis is more than a beginning in this difficult field; it pointed the way, however, for many investigations which followed, and for the future.

All tannins do not behave similarly in their action upon skin, nor in their response to various reagents. For this reason many attempts have been made to classify them. One of the earliest attempts was to divide tannins into two classes, the pyrogallol and the catechol. This was based upon the fact that when the natural tannins were heated to 180-200°, they yielded either or both of these products. The pyrogallol-yielding tans usually gave a blue coloration with ferric salts and the catechol tannins a green. This differentiation method has not proved satisfactory, if for no other reason than that some commercial tannins may contain both groups. More satisfactory and comprehensive classifications have been suggested by Perkin and Everest and by Freudenberg.

Perkin and Everest[80] divided tannins into three groups:

- α, Depsides or gallotannins
- β, Diphenylmethylolids or ellagitannins
- γ, Phlobotannins or catecholtannins.

These three groups respond to treatment with various reagents as follows:

Treatment with Ferric Chloride:

(α) Gives blue coloration
(β) Gives green coloration

Treatment with boiling dil. H_2SO_4:

(α) Forms gallic acid
(β) Precipitates ellagic acid
(γ) Precipitates phlobophenes or "reds"

Treatment with Bromine:

(γ) Yields precipitate

Treatment with Pine Wood and HCl:

(γ) Gives phloroglucin reaction, while (α) and (β) do not

Treatment with Diazobenzene Chloride:

(γ) Gives precipitate indicating presence of phloroglucin or resorcin groupings, while (α) and (β) do not

Fusion with Alkali:

(α) Yields gallic acid and traces of pyrogallol
(γ) Yields protocatechuic acid

Heating in Glycerol:

(α) Forms pyrogallol
(γ) Forms catechol

Treatment with HCHO and HCl:

(γ) Gives complete precipitation; the others do not

Treatment with Lead Acetate in $CH_3 \cdot CO_2H$ solution:

(α) Gives precipitation while (γ) does not

Freudenberg[36] has classified the tannins into two main groups. These groups and their subdivisions are:

(A) **Hydrolyzable tannins,** in which the benzene nuclei are united to a larger complex by means of the oxygen atoms.

(A_1) Mutual esters of phenolcarboxylic acids or with other hydroxy-acids (Depsides).

(A_2) Esters of phenolcarboxylic acids with polyatomic alcohols and sugars.

(A_3) Glucosides. Gallic acid predominates as the phenolic component of this group. There exists also the extraordinary distribution of combined caffeic acid as well as the presence of a new phenolcarboxylic acid in chebulinic acid. There are also the ellagic acid glucosides. Probably the most important criterion for inclusion in this group is the ability to be split into simple components by means of hydrolyzing enzymes, such as tannase and emulsin.

(B) **Condensed tannins,** in which the nuclei are held together by means of carbon linkages. These tannins, unlike the hydrolyzable, cannot be split into simple components by enzymes. They are sometimes precipitated by bromine. They condense to higher molecular weight tannins ("reds") when

acted upon by oxidizing agents or strong acids. Under drastic treatment, such as by alkalies, the carbon skeleton is disintegrated, and if phloroglucinol is present, it is dissolved out, and the residue of the molecule is converted mainly into phenolcarboxylic acids.

(B_1) This group consists of simple ketones, such as hydroxybenzophenones and hydroxyphenylstyrl ketones.

(B_2) The phloroglucinol and benzene nuclei are present in equimolecular proportions. This class includes the catechols, with their corresponding tannins and "reds." This is the most important class of commercial tannins.

(B_3) Not much is known as to the chemistry of this class, or as to their really being jointly condensed systems. However, in common with the B group of condensed tannin, they are precipitated by bromine and condensed to form "reds." They contain no phloroglucinol nucleus. It may be that the hydroxycinnamic acids are characteristic components. Caffeic acid is readily changed into condensation products of the nature of reds.

Freudenberg's classification has come into rather wide use. The principal commercial tannins are thereby grouped as follows:

Hydrolyzable tannins comprise chestnut and myrobalans, while quebracho, mangrove, wattle, hemlock, and gambier are of the condensed type. Valonia and Chestnut oak bark contain both types of tannin.*

Russell[93] has made an extensive survey of our present knowledge of the chemistry of the tannins. He points out that the most important tannins, including those which are commercially employed, belong to the phlobotannin group; he suggests, in view of this, that an extensive tannin classification may eventually be found to be unnecessary, and that tannins which do not produce phlobaphenes may come to be regarded as exceptional substances which happen to have tanning properties. Russell, Tebbens, and Arcy[94] have recently reported the preparation of four pentagallates: *β-d*-glucose pentagallate, *d*-mannose pentagallate, *β-d*-glucose diethylmercaptal pentagallate and aldehydo-*d*-glucose pentagallate. They state that all these compounds have tanning properties, forming leather which is strictly comparable in color and quality with that produced by natural gallotannin.

Nontannins. The proportion or percentage which the tannin represents of the water soluble matter in a tanning material is termed the "purity" of that material. This value varies greatly. Thus quebracho extract when analyzed by the "official" method, to be described in another section, may show 90.0 per cent of its water-soluble content to be tannin and only 10.0 per cent to be nontannin, whereas gambier may show 57.0 and 43.0 per cent respectively. The proportionate amount of nontannins, and their chemical character, greatly influence the behavior of the accompanying tannin toward

* For further information regarding classification of the tannins according to the method of Perkins and Everest and also of Fruedenberg, see the second edition of this monograph.

the hide substance. As a general rule—though not strictly so—the higher the purity of the material, the greater its astringency, and hence the greater its rate and amount of tannin fixation.

The nature of nontannins varies with the tanning materials themselves; the former may change in nature as a function of fermentation, oxidation, or other influences. As a general rule, the nontannins are composed mainly of carbohydrates, mineral salts, and organic acids, such as lactic, acetic, etc., while some tanning materials may contain gallic acid, pyrogallol, and similar bodies. The influence of the nontannins, as indicated above, is of importance in regulating the tanning behavior of the tan liquor.

Clarke and Frey[25] have determined the reducing sugars present in various commercial tanning materials, which they describe as not necessarily representing the "true sugar" content of the materials but are: "a measure of the Fehling's (reducing) power in terms of dextrose." These results are shown in Table 234 as the percentage of dextrose calculated on the tannin content of the several materials.

Table 234

Algarobilla pods	6.4	Quebracho wood	1.3
Chestnut wood		dry extract	1.0
liquid extract	10.2	Sumac leaves, domestic	12.9
dry extract	11.3	liquid extract	13.5
Divi-divi pods	6.4	Sumac leaves, Sicilian	11.3
Hemlock bark		liquid extract	12.9
dry extract	20.1	Valonia	
Mangrove bark	2 3	(beards)	5.6
Mryobalan nuts	6.8	(cups)	7.0
Chestnut oak bark		Wattle bark	
dry extract	18.9	(1)	2.8
California tan-oak bark	11.2	(2)	7.2
liquid extract	4.1	dry extract	7 8

The presence of furfural, pentose, and uronic acids has been proved in the case of a number of tanning materials. This subject has been investigated and discussed by van Gijn and van der Waerden,[124] by Reed and Schubert,[88] and by Phillips.[81]

Organic acids of various types are present in tan liquors and will be discussed later. But since they are an important nontannin constituent, it may be well to note the amounts contained in liquors made from typical commercial tannins as shown in Table 235. The values for cutch, oak bark extract, hemlock extract, and chestnut wood extract were obtained by Cameron and McLaughlin,[18] and those for myrobalans, ordinary quebracho extract, and wattle extract by Wilson,[133] who employed the analytical methods devised by Cameron and McLaughlin. The results are expressed as ml of 0 1N acid present in 100 ml of liquor of 30° barkometer (S. G. 1.030).

Cutch, hemlock, quebracho, and wattle are all of the condensed type;

Table 235

	Lactic Acid	Acetic Acid	Gallic Acid
Cutch (mangrove)	9.60	1.60	0.40
Hemlock	2.86	2.04	8.36
Quebracho (ordinary)	0.52	2.73	3.82
Quebracho (sulfited)	0.74	...	3.26
Wattle	0.04	0.23	1.32
Chestnut	1.00	8.40	40.76
Myrobalans	5.00	0.42	31.20
Oak bark	4.96	2.52	14.36

chestnut and myrobalans are of the hydrolyzable type with high gallic acid value, while oak bark is a mixture of both types.

The amount and kind of mineral salts present in tan liquors varies with the tanning materials employed, and this, in turn, varies with the locality in which the materials grow. Mineral salts present are also a function of the composition of the water used in dissolving the tanning materials at the tannery, and of the calcium salts brought into the early liquors by the hide or skin.

Insolubles. Vegetable tannin solutions always contain matter which is insoluble in water. The amount of such insolubles varies with the nature of the tannin material itself and with conditions such as temperature, concentration, pH value, the presence of hydrolyzing enzymes, etc.

In the case of the hydrolyzable tannins the insolubles are principally composed of chebulinic and/or ellagic acids. Chebulinic acid is readily soluble in hot water, but only sparingly so in cold. Thus it may be extracted from a material which is leached with hot water, or dissolved when an extract is treated with hot water, but it will be precipitated when such solutions have cooled. Ellagic acid is highly insoluble in water but is soluble when it is combined with some soluble substance, such as glucose; but if such soluble compound is acted upon by mold enzymes or by acids, the ellagic acid may be split off and then precipitated.

The insolubles of the condensed tannins are mainly phlobaphenes formed by the condensation of tannin molecules. The composition of the phlobaphenes has been specially investigated by Bergmann and Pojarlieff.[9] These authors have also studied the changes induced in the structure of quebracho tannin when it is given the so-called sulfiting treatment to solubilize the large amount of phlobaphenes it naturally contains.

Botanical Function of Tannin

Despite the very wide occurrence of tannin in plant life, botanists are still unable to explain its origin or function. Some botanists believe that it increases the resistance of plants to fungus diseases.

Analytical Determination of Tannin

The first problem confronting leather chemists was to devise a method of analysis whereby the amount of tannin present in a raw vegetable material, an extract, a tan liquor, or the fixed and unfixed tannin in leather could be accurately determined. Many schemes of estimation by precipitation or oxidation of the tannin bodies by purely chemical means were unsuccessfully tried. They proved unsuccessful not merely because of the diversity in composition of the tannins themselves nor because of the impurities always associated with them, but because the real tanning value of a material is determined by the extent of its reaction with hide substance itself.* Recognizing this fact, chemists spent many years in perfecting methods by which hide-substance tannin fixation could be accurately and rapidly determined. Two such methods have been evolved and are in current use.

The "official" method of The American Leather Chemists Association, which is most widely employed in the United States and, with slight modifications, in many foreign countries as well, is briefly as follows:

The tannin solution of approximately proper concentration is agitated for ten minutes with the proper amount of finely ground and divided hide substance, in which form the latter is known as "hide powder." (The hide powder is prepared from steer or cow hide which has passed through the processes of soaking, liming, unhairing, and careful deliming before it is dried prior to grinding.) Such finely divided hide substance presents great reactive surface and is easily and quickly permeated by the dilute tannin solution. The hide powder is tanned during the shaking and becomes leather, and only the unabsorbed "nontannins" remain in the solution. The weight of these is determined and deducted from the weight of the soluble matter in the original solution; the difference between the two weights is termed "tannin."

This method has been in use for many years and has proved valuable both as a means of following tannery operations and as a basis for the purchase of tanning materials. Its main defect is the fact that it tells nothing as to how firmly or loosely the tannin has been fixed by the hide powder—in other words, it does not distinguish between reversible and irreversible tannin fixation. In view of this, Wilson and Kern[127] suggested a method of tannin estimation which is presumed to estimate only the irreversibly fixed or combined tannin. Their method consists essentially of shaking the mixture of hide powder and tan solution for six hours and then washing the tanned powder with distilled water until the wash water shows no test for tannin with gelatin/sodium chloride solution. The washed powder is then dried, and its increase in weight is expressed as the percentage of irreversibly fixed tannin derived

* This does not mean, however, that adequate "chemical" methods may not be evolved in the future; such methods would be very useful.

from the tan liquor. Both methods are empirical, and each has advantages and disadvantages, which will be further considered when the nature of tannin fixation is discussed. Meanwhile, the comparative results of the two methods when applied in the analysis of typical commercial tannins is shown in Table 236.

Table 236

Material	Per cent Tannin	
	Official Method	Wilson and Kern Method
Quebracho extract	68.01	47.41
Hemlock bark	10.06	6.17
Chestnut wood extract	25 80	11.90
Oak bark extract	24 20	12.88
Sumac extract	25.56	9.61
Gambier extract	24.95	7.79

Page has amplified the method of Wilson and Kern to include three values: (1) firmly fixed or combined tannin, (2) matter termed "combined water solubles," and (3) "free water solubles." These values and their derivation are discussed on page 605.

Differentiation of Tannins in Mixtures, and Preferential Absorption by Hide Substance Therefrom

Modern vegetable tanning is generally accomplished with a mixture or blend of several different tanning materials. The tanning behavior of such mixtures is very probably a function, among other factors, of the characteristics of the individual component materials and of the manner in which each influences the others. For this reason accurate methods for differentiating tannins in admixture are greatly needed. Much effort has been spent in devising such methods, a detailed account of which may be found in the very excellent third edition of "Procter's Leather Chemists' Pocket-Book" by Atkin and Thompson.[5] But as they point out, many of the tannins are so similar in composition that differentiating them in mixtures becomes difficult.

In 1910, Neuner and Stiasny[69] studied the diffusion of mixtures of tannin through parchment. These blends were composed of typical materials of the catechol and pyrogallol groups. Catechol tannins are usually more or less completely precipitated by boiling with formaldehyde and hydrochloric acid, whereas the pyrogallol tannins are not thus precipitated. Utilizing these precipitation methods, they determined the approximate composition of the diffused solution. Their results indicated that sometimes the dialyzate conformed in composition to the original, undialyzed mixture; but this was not always true, indicating that one tannin affects the behavior of another.

Grassmann, Miekeley, Schelz, and Windbichler[42] tanned pelt with varying mixtures of mimosa extract and sulfite cellulose and then analyzed the unwashed leathers for sulfur. Since the sulfite cellulose contains sulfur and

the mimosa does not, to any appreciable extent, it was possible to compute accurately the proportion of the two materials absorbed by the leather in relation to the original mixture. The results were as follows:

% sulfite cellulose in tanning blend	0	20	50	80	100
% sulfite cellulose in tannin absorbed	0	11	23	42	100

The sulfite cellulose was not absorbed in relation to the blend.

The latest investigation of this subject is that of Blockley, Spiers, and Florin,[13] who studied the behavior of mixtures of chestnut and quebracho, chestnut and pine bark, and chestnut, quebracho, and pine bark when used to tan hide powder. They employed a modified formaldehyde/hydrochloric acid method to differentiate the type of tannin in the residual tan liquors. Their results indicate that chestnut tannin is absorbed some 20 per cent faster than quebracho from a chestnut/quebracho mixture and 40 per cent faster than pine bark tannin from a chestnut/pine bark mixture. In commenting upon these results, they state that the preferential absorption of chestnut shown is not as great as might be expected, but that the potentially different absorption rates may be modified by the fact that when tannins are mixed, they then function at the nontannin concentration of the mixture and at a common pH.

We cannot, of course, be sure that experiments with hide powder and those wherein tannage is performed with very dilute liquors (0.5 per cent tannin) for the short period of one hour, are typical of tannery operations in which hide itself is employed with much stronger liquors and much longer time. For these reasons it may be hoped that further studies under actual tannery conditions will be made. Such investigations would be greatly enhanced by information showing particle-size changes as a function of admixture. But this knowledge will have to await the evolution of a dependable method for particle-size determination.

Molecular Weights of Tannins and Their Bound Water

In 1861 Thomas Graham called attention to the fact that the tannin molecule must be large, since its diffusion velocity is so much less than that of sodium chloride. This observation was followed by a number of determinations by different workers of the molecular weight of gallotannic acid. When the molecular weight of gallotannic acid dissolved in an aqueous solution was determined, the values ranged from 521 to 3700; when dissolved in glacial acetic acid, they were 340 to 1322; and when dissolved in acetone, 441 to 705. A great need existed for accurate molecular weight values for various tannins. This subject has been investigated in a series of painstaking studies by Humphreys[53] and by Douglas and Humphreys.[31]

Humphreys determined the freezing point of 20° barkometer liquors,

which were first allowed to settle and were then filtered. A modification of the Hortvet cryoscope was used, and the freezing points were determined by freezing 20 ml of the sample and calculating the molecular weights in the usual manner. The results are shown in the first column in Table 237. In a later study, Douglas and Humphreys pointed out that the properties of the actual tannins may be influenced by associated nontannin matters. They therefore repeated the determinations of Humphreys, using the same methods, but including the molecular weight values for the same liquors, which had first been purified by electrodialysis against distilled water through Grade 300 Cellophane (0.0008 inch thick). Both values are shown in the table, to which we have added the "purity" of the liquor, that is, the percentage of the soluble solids which is tannin, before and after electrodialysis.

Table 237

Material	Mol. Wt [44]	Mol Wt [31]	Mol Wt after dialysis	Purity before dialysis (%)	Purity after dialysis (%)	Bound Water per gram × 1000 of T S / Mol Wt
Paradol*	132					13.00
Old Myrobalan liquor (a)	181					7.13
New Myrobalan liquor (b)	256	340	1917	66	86	3.28
Algarobilla	237					4.77
Mimosa bleach	258					3.84
Quebracho bleach	268					2.83
Gambier						
(a)	308	324	520	63	87	3.12
(b)	320					3 88
(c)	330					3.00
Valonia	362					3.62
Chestnut						
(a)	447	462	1545	74	84	1.02
(b)	557					1.30
Mimosa						
(a)		603	1570			
(b)	565	432	1704	70	95	1.24
Quebracho						
(sulfited)	910	369	763			1.92
(ordinary)	1950	1159	2421	83	95	0.83
Tannic acid	1302	1445	3434			0.45

* Paradol is a synthetic formaldehyde-phenol tanning material.

In commenting upon their results, Douglas and Humphreys pointed out that their cellophane membranes retained only those molecules whose weight exceeded 500. They concluded that the molecular weight of most tanning materials is probably at least 2000, and that the molecular weights found were usually much greater than would be indicated from the formulas suggested by investigators of their chemical constitution. Fischer and Bergmann's formula for pentadigalloyl glucose, for example, was only 1700, whereas they found the molecular weight of dialyzed tannic acid to be 3434. They sug-

gested, therefore, that actual tannin molecules were built up from a number of simpler compounds.

Humphreys attempted to derive the "bound water" values of various tannins. He employed Gortner's sucrose reference method, the principle of which is as follows:

A known amount of sucrose is added to a solution containing a known amount of water, and the freezing point of the solution is determined. If all the water present is "free," the freezing point depression will be of the theoretical value; if part of the water is bound (*i.e.*, to the solids of the tan solution, in this case), the freezing-point depression will be greater than the theoretical, since the actual glucose concentration will be greater. From these values the bound water may be calculated.

The bound water was thus determined for the materials appearing in the first column of Table 237; and they are shown in the last column expressed as the observed bound water divided by the molecular weights given in the first column. These values indicate that the more astringent materials contain much less bound water than the less astringent. It is to be hoped that bound water values will be obtained for dialyzed and purified materials.

Humphreys cautioned against too much dependence being placed in the bound water values shown, because the added sucrose may have united in some manner with the liquor constituents and thus affected their behavior. We would add that the accuracy of all the various freezing-point methods for bound water determination is somewhat questionable. The freezing point of a solution is actually the temperature at which the solid and liquid phases are in equilibrium, and any interference with the attainment of such equilibrium may lead to fictitious values.

Molecular weight studies emphasize the need for a dependable method for the determination of particle size.

Particle Size of the Tannins

While the colloidal nature of the tannins has long been known and was recognized by Thomas Graham, the founder of colloid chemistry, and by many of the early leather chemists, the great importance of degree of dispersion was first clearly formulated by G. W. Schultz[95] in 1921. In a discussion of methods of tannin analysis, Schultz stated: "This brings us to the conclusion that in a solution of tanning material we have tannin in all degrees of dispersion, varying from a molecular dispersion to one of suspension dimensions, and it is evident that the proportion of the least dispersed to the greatest depends upon the concentration."

Since 1921 a number of efforts have been made to determine dispersion values and how these are affected by different tanning conditions and factors; and in our opinion there is no problem in vegetable tanning that is of greater

importance. A dependable quantitative method whereby dispersion values could be determined would probably not only greatly simplify the control of commercial tanning, but would make possible great advances in scientific studies and go far in aiding the synthesis of greatly needed tanning agents.

Dispersion studies have followed two general lines: precipitation with salts and by ultrafiltration.

Stiasny and Salomon[105] precipitated tannin from various tanning solutions with increasing additions of sodium chloride. The principle involved was the assumption that the larger particles were precipitated by the lower salt concentrations and that the smaller the particle, the higher was the salt concentration required for salting out. The proper amount of powdered salt was added to 100 ml of the tannin solution, which was shaken until the salt was dissolved, allowed to stand one hour, and filtered by suction. To the filtrate (which contained the unprecipitated matter) an increased addition of salt was added, and these operations were repeated until salt saturation was reached. The precipitated fractions were then separately dissolved in distilled water at 50°, and their tannin content was determined by means of the Löwenthal oxidation method, which was also employed in determining the tannin content of the final salt-saturated solution. The results are shown in Table 238, giving the percentage of the 100 per cent tannin originally present which was precipitated by each of the various salt concentrations.

Table 238

Material	Solids Solution Strength (%)	% Sodium Chloride Added 8	18	20	26	32	Total Tannin ppt (%)	Unppt. Tannin in Residue (%)
Tannin (Merck)	7.50	clear	17.1	..	32 4	17.2	66.7	32.2
"	3.50	"	4.4	..	35.3	11.4	51 1	46.4
"	1.25	"	4.6		34.0	11.0	49.6	49.4
Quebracho	1 00	23.6		25.2	...	24.4	73.2	26.8
Oakwood	1.00	20.7	..	15.6	...	8.6	44.9	54.5
Chestnut wood	1.00	20.6	.	10.5	...	9.8	40.9	60.0
Oak bark	1.00	20.1	..	14.9	12.8	7.3	55.1	36.1
Mimosa	7.50	8.3	..	22.1	12.3	1.3	44.0	56.0
"	0.85	10.0	...	14 3		19.0	43.3	56.7
Sumac	1.00	7.6	...	16.7		14.7	39 0	57.1
Mangrove	6.80	17.6	..	26.7	.	15.9	60.2	37.8
"	1.70	11.7	..	17.7	.	17.7	47.1	52.4
Valonia	1.00	4.6	8.8	...	..	9 9	23.3	75.6

They found that the total amount of tannin salted out increased with the aging of the solution. The addition of sugar had no effect upon the total precipitable fraction, but the addition of gallic acid decreased it. The tannin from a number of materials was secured in a purer form by precipitation with neutral lead acetate, and the lead/tannin compound was then decomposed. Such purified tannin showed much higher degrees of dispersion than did the original materials. Salt precipitations seemed to follow the Schulze-

Hardy valency law: the precipitating power of the cation increased with increasing valence, that of the anion decreased with increasing valence, and that of the chlorides, NaCl, KCl, and NH_4Cl, was in the order given, NaCl being most potent.

Stather and Schubert[103] have investigated a number of tanning materials by the salting-out method, essentially employing the methods of Stiasny and Salomon and in general confirming their findings.

We ourselves have employed the salting-out method for control purposes over a considerable period of time. We have found that changes in the tanning character of vegetable tan liquors can be roughly detected and followed by this method. But we have not had great success with the Löwenthal procedure which the authors quoted above employed, because of the difficulty in securing satisfactory check results on duplicate determinations. We have therefore dissolved the precipitated tannin in hot water at 85°, and after cooling, have dialyzed it in a cellophane bag against running tap water for 24 hours, thereby removing the salt and other nontannin matters present. The dialyzed liquor is then analyzed with hide powder according to the official method.

The second method, and the theoretically sounder one, for dispersion estimation is that of ultrafiltration. The first successful attempt in this direction was made in 1923 by R. J. Browne,[16] working in the Procter Research Laboratory at Leeds. Browne used a modified Bechhold apparatus and membranes prepared by soaking filter paper in collodion dissolved in a 50-50 alcohol-ether solution. The impregnated papers were then placed in water and washed until free of alcohol-ether. They were then standardized by measuring their permeability to water under variable pressures. (The actual size of the membrane pores was not determined.) Employing membranes which prevented the passage of tannin, Browne examined a large number of tan solutions of analytical strength; the percentages of tannin found, compared with tannin secured by the official hide powder method, are noted in Table 239. The ultrafiltrate obtained (which constituted the nontans) was

Table 239

Material	Per cent Tannin	
	Official Method	By Ultrafiltration
Wattle extract	62.90	61.80
Liquid chestnut wood extract	25.10	25.20
Myrobalans	51.30	51.00
Gambier (cube)	21.00	20.40
Hemlock bark	23.30	24.30
Sumac	27.10	26.90
Ordinary quebracho	25.50	25.80
Valonia, beard	46.40	46.50
Tari pods	47.70	47.80
Tannic acid	82.50	79.10
Gallic acid	50.40	0.00

dried *in vacuo* to constant weight. An aliquot of the original unfiltered solution was similarly evaporated. The difference between the two gave the tannin plus unsolubles. Deducting the insolubles (as determined by the official method) yielded the tannin. The agreement in tannin values by the two methods is quite remarkable, and the zero value of the ultrafiltered gallic acid solution is interesting. By ultrafiltration, Browne was also able to separate quantitatively the tannic acid in a solution containing equal parts of tannic and gallic acids.

Thomas and Kelly[118] have criticized Browne's method on the basis of the facts that duplicate membranes are difficult to prepare, that reactions may occur between membrane and solution being filtered, and that the filtration value may vary with fluctuations in pH of the solution. Browne pointed out, however, that in view of the results of his studies and the purposes for which they were made, these criticisms cannot be of great moment because of the remarkable checks between his ultrafiltration results and those by the official method.

Stather and Schubert [103] have also studied the tanning properties of various materials after dialysis through parchment membranes for varying time periods. They found increased tannin absorption by hide powder as dialysis proceeded, that is, as the proportion of large particles in the liquor was theoretically increased, and as the purity of the liquor was raised.

The latest ultrafiltration study is that of Compton,[28] who has studied the ultrafiltration of ordinary quebracho solutions of varying concentration, with and without the addition of syntan. His membranes were prepared from an ethyl alcohol-ether-acetone-amyl alcohol solution of Parlodion. He found no uniformly direct relation between the pore diameter in millimicrons of membrane and the amount of tannin passing through, when duplicate determinations were made with the same solution, using separate membranes.

There can be no doubt but that the problem of devising a dependable method for degree of dispersion is one of considerable difficulty; but in view of the importance of the information it would yield, it is to be hoped that leather chemists will continue to attack it.

The Electrical Charge of Tannins

An important part of current vegetable tanning theory is based on the assumption that the tannins are negatively charged, and to an appreciable extent. As will be shown below, this theoretical interpretation does not rest upon very sure experimental ground even today.

The first experimental study of the electrical charge of tannins seems to have been that of Ricevuto[89] in 1908. Ricevuto's results are confusing, doubtless due to faulty technique. The same remarks apply to Grasser's[41] investigations. The first experimentally sound studies are those of Thomas

and Foster[119] published in 1922 and 1923, long after tanning theories based on negatively charged tannins had been announced. Thomas and Foster measured the potential difference between the colloidal tannin particles and their aqueous dispersion medium, employing the U-tube electrophoresis technique of Burton. First they studied eight different commercial tannins, in such concentrations as to give 1.0 per cent tannin. The results are shown in Table 240, in which the order of conductivities is also given. This table

Table 240

Material	Potential Difference (volt)	Decreasing Conductivity
Gambier (cube)	−0.005	Sumac
Oak bark	−0.009	Gambier
Chestnut wood	−0.009	Oak bark
Hemlock bark	−0.010	Larch bark
Sumac	−0.014	Hemlock bark
Larch bark	−0.018	Chestnut wood
Osage orange	−0.018 (?)	Osage orange
Ordinary quebracho	−0.028	Quebracho

unfortunately does not include pure tannic acid. They then studied the effect of concentration and of the addition of acid to ordinary quebracho extract, as shown in Tables 241 and 242.

Table 241

Concentration (Gms Solids per l)	Potential Difference (volt)
32	−0.024
16	−0.028
8	−0.029
4	−0.030

Table 242

16 Gms Solids per l		
Ml 0.1N HCl added per l	pH	Potential Difference (volt)
0	4.75	−0.024
10	3.98	−0.014
15	3.58	−0.010
20	3.37	approx. 0.000

Thomas and Foster did not include the pH values of the quebracho solutions to which acid was added as shown in Table 242. The pH values shown were determined by us with a glass electrode.

In order to determine the effect of removal of nontannins upon the value of electrical charge, they selected five materials which were dialyzed through collodion sacs against distilled water, and their potential differences were determined, as shown in Table 243.

Sokolov and Kolyakova[97] have reported potential differences of −0.0106

Table 243

Material	Concentration (Gms per l)	Time (Hrs)	Dialysis Volume Increase to ml (from 200 ml)	Potential Difference (volt)
Ordinary quebracho	16.0	60	415	−0.033
Osage orange	16.0	24	370	−0.024
Sumac	16.0	24	460	−0.026
Gambier (cube)	32.8	24	390	−0.029
Hemlock bark	...	24	..	−0.024

volt for spruce extract and −0.0095 volt for oak bark extract, both of which had been purified by electrodialysis. They found that the voltage approached zero in all cases when acids were added. Values for unpurified natural extracts were: oak bark −0.0079 volt, quebracho −0.0074, spruce −0.0069, and willow −0.0045. They did not find the relation between potential difference and astringency which Thomas and Foster noted.

In a very important article on the nature of vegetable tanning, Burgenberg de Jong[17] considered the subject of electrical charges of tannins. He reported making many cataphoretic experiments with *pure tannic acid* in a Burton apparatus (the same as that employed by Thomas and Foster) and that he was unable to find any indication of a capillary electrical charge. He then examined the tannic acid by viscosimetric means, on the basis of von Smoluchowski's suggestion that a charged colloid must show a higher viscosity than an uncharged one. Since he could find no charge on the tannic acid by either electrophoretic or viscosimetric means, he concluded that the charges on commercial tannins found by Thomas and Foster were derived from their associated impurities and not from the tannins themselves.

As indicated above, and as will be discussed later, the contention that electrical charges on tannins are of fundamental importance does not rest upon any sound foundation as yet. Further studies of this important point should be made.

Isoelectric Points of Tannin

The "isoelectric point," formulated by W. B. Hardy in 1900, indicates the pH value at which a substance is electrically neutral. Since much of the theoretical reasoning regarding the mechanism of vegetable tanning involves the neutralization of electrical charges, the isoelectric values of the tannins become a matter of importance. Consequently, Thomas and Foster[119] have attempted to determine them. This was done by observing the migration of various tannins at different pH values in an electrophoresis U-tube. Experimental difficulties necessitated dissolving the tanning materials in a citrate buffer solution and then adjusting the solution to the desired pH values. The results were not entirely satisfactory, but they indicated (1) that the isoelectric points of hemlock, oak, wattle, sumac, and gambier lie between pH 2.0 and 2.5; (2) that at pH values below 2.0, these tannins became positively charged.

The cataphoretic studies which Sokolov and Kolyakova[97] made with purified quebracho, oak bark, and spruce bark tannins led them to conclude that these tannins have no definite isoelectric points. They reported acid dissociation constants for oak bark tannin of $K_1 = 6 \times 10^{-5}$, $K_2 = 10^{-8}$ and for spruce bark tannin $K_1 = 2.5 \times 10^{-5}$, $K_2 = 2 \times 10^{-10}$. K_1 is attributed to a carboxyl group and K_2 to a phenolic.

Diffusion of Tannins into Hide or Skin

In 1911, A. W. Hoppenstedt[52] poured 5.0 per cent gelatin solution into test tubes; when the gelatin had cooled and become a jelly, he covered the gelatin in the tubes with solutions of various tanning materials, containing 4.0 grams tannin per liter. The rate of diffusion of the various materials as a function of time was noted, and the order of increasing diffusion was found to be: mangrove bark, ordinary quebracho, hemlock bark, algarobilla, valonia, oak bark, myrabolans, chestnut wood, gambier, divi-divi, and sumac. This seemed to be roughly the order of decreasing astringency. Thomas[108] repeated these experiments and generally confirmed Hoppenstedt's findings; but since neither of these investigators had determined or controlled pH values, Wilson and Kern[128] repeated their experiments in order to ascertain the effect of varying pH values. The 5.0 per cent gelatin solutions were brought to pH 2.5 with tartaric acid, and a range of pH values of 2.5 to 11.0 was secured by addition of the required amount of sodium hydroxide. The pH values of solutions of gambier and of ordinary quebracho, containing 10 grams solid matter per liter, were adjusted in exactly the same manner. Solutions of these materials were then poured into the test tubes containing the 5.0 per cent gelatin jelly, the pH value of jelly and accompanying tan solution being identical in each tube. The diffusion differences noted after 96 hours were very great. Gambier began to diffuse at pH 3.0 and reached a maximum diffusion at pH 6.0, the diffusion decreasing with increasing pH values beyond 6.0. The diffusion of quebracho began at pH 4.7 (the isoelectric point of the gelatin) and increased continuously to pH 11.0.

The above experiments were all made with gelatin jelly, the structure of which is very different from that of hide or skin as used in tanning. Consequently, Mezey[67] studied the rate at which tan liquor diffused into pieces of calf skin which had been unhaired and delimed. Solutions of various materials were prepared to contain from 0.5 per cent to 8.0 per cent solids, and the time of tanning was from 0.5 to 24 hours. Diffusion of the tannin was measured by sectioning the leather with a microtome and staining the sections with potassium bichromate, the depth of tannin penetration being measured under the microscope by means of a micrometer. The bichromate stained the tanned portion brown to a brownish black coloration. The slides showed that the rapidity of diffusion could not be correlated with astringency. Chestnut and ordinary quebracho penetrated more rapidly than sumac. This statement refers to dilute solutions. Mezey believes the general opinion that the so-called astringent tans penetrate slowly is correct only when it refers to concentrated solutions, and that the slower diffusion in concentrated solutions is due to over-tanning of the skin's surfaces ("case-hardening") which prevents further diffusion: that is, the diffusion power of these tans, as such, is not necessarily low.

Stather[98] has also studied the diffusion of various tannins into pelt, essentially employing the same methods as Mezey's. He has found that the tannin diffusion from dilute solutions (0.75 per cent tannin) may be expressed by the formula $E = K\sqrt{T}$, where E is equal to the diffusion and is proportional to the square root of time of tanning, and where K is the diffusion constant. This constant has approximately the same value for 6 or 24 hours but tends to increase with longer time. Taking the diffusion constant of gambier as 100, and relating the other materials thereto, the various materials may be arranged into four groups, as follows: (1) Pine bark, 28. (2) Mangrove, 46; myrabolans, 50; sumac, 52. (3) Ordinary quebracho, 63; chestnut, 66; oak wood, 69; algarobilla, 74. (4) Mimosa, 78; sulphited quebracho, 83; valonia, 89; and gambier, 100.

There seemed to be no relation between penetration and ratio of tans to nontans (astringency) as Hoppenstedt, Thomas, and Wilson had suggested. The various materials were also all brought to pH 4.0, and their diffusion rates were determined, when they all showed essentially the same as above. But the diffusion differences between the various materials were not so apparent when stronger solutions, *i.e.*, 4.5 per cent tannin, were employed; neither did the suggested diffusion formula given hold as well.

The diffusion or penetration of the various tannins noted above does not necessarily coincide with blended liquors in tannery practice, where local conditions have great influence; and we do not believe diffusion studies can be profitably extended until a dependable method for particle-size studies has been evolved.

Conversion of Nontannins into Tannin

It has been found possible, under certain conditions, to change substances which are considered typical nontannins into substances which react with hide substance and hence would be termed tannin. The mechanism of this phenomenon is not clear, nor is the transformation likely to occur during the tanning process.

Wilson and Kern[126] tanned hide powder with gambier extract and then washed it with distilled water. They collected the later portions of the wash water and found them to contain no tannin when tested with gelatin/salt. But upon concentrating the washings *in vacuo*, they did give a tannin test, and continued to do so even when the concentrated solution was diluted back to its original volume. They suggested that oxidation, condensation, or polymerization might be involved. They suggested also that gallic acid might be converted to digallic and that a polymerized form of digallic acid would have tanning properties. They found that pure gallic acid gives no tannin test with gelatin/salt reagent but does so if the solution has been boiled. They also found that a nontannin solution may be made to

give a tannin test by the passage of oxygen through it, or by long exposure to air.

Meunier[66] has also shown that while gallic acid has no tanning action, it does have if oxidized.

On the other hand, G. W. Schultz[95] has suggested that no chemical change is necessarily involved in these conversions (to which he was the first to call attention) but, more probably, that a change in dispersion degree occurs. He prepared a solution of chestnut extract which contained 1.8 grams tannin in 8000 ml water. At this great dilution no tannin test with gelatin/salt was obtained. When the 8000 ml were concentrated *in vacuo* to 200 ml and again diluted to 8000 ml, a decided tannin test was secured. If, however, the 200-ml concentrate was further evaporated to the consistency of a thick syrup and diluted back to the 8000 ml, no tannin test was obtained. Schultz explained these phenomena by the assumption that the tannin in the first dilution became so highly dispersed that it was incapable of precipitating gelatin. By heating and concentrating, the tannin became associated and was more coarsely dispersed after subsequent dilution. When concentration was carried to the syrupy state, the tannin was brought, possibly by peptization, to its original high degree of dispersion and so could not precipitate gelatin when diluted.

This subject is one illustration, of which we shall find many, of the great need in leather chemistry for a dependable method of determining the particle size of tannin bodies.

Effect of Temperature on Tannin Fixation

Tanners and leather chemists have long known the great importance of the temperature factor in vegetable tanning. As tan liquor temperatures fall below 21°, the rate of tanning rapidly decreases, and flat leather usually results; when temperature rises above 27°, the rate of tanning usually increases, but undesirable leather qualities may ensue. Despite the importance of the subject, it received no quantitative investigation before the studies of Thomas and Kelly in 1929.

Thomas and Kelly[120] tanned 2.0 grams hide powder with a 200 ml solution containing 8.0 grams total solids for varying time periods and at different temperatures. The tanned powders were then washed free of soluble matter and were dried and analyzed. Their experiments included wattle, oak bark, gambier, and quebracho extracts. Since the tannin-fixation curves of all four materials are of quite similar shape, we have reproduced those of quebracho only in Figure 164.

The great effect which temperature has on tannin fixation is illustrated by these curves. Thomas and Kelly pointed out that, in addition to ordinary temperature effects in chemical reactions, the influence of temperature upon

the degree of dispersion of the tannin particles must be considered. Their experiments were made with hide powder. Merrill[63] has repeated them using bated calf skin and a regular tannery blend of tanning materials. The

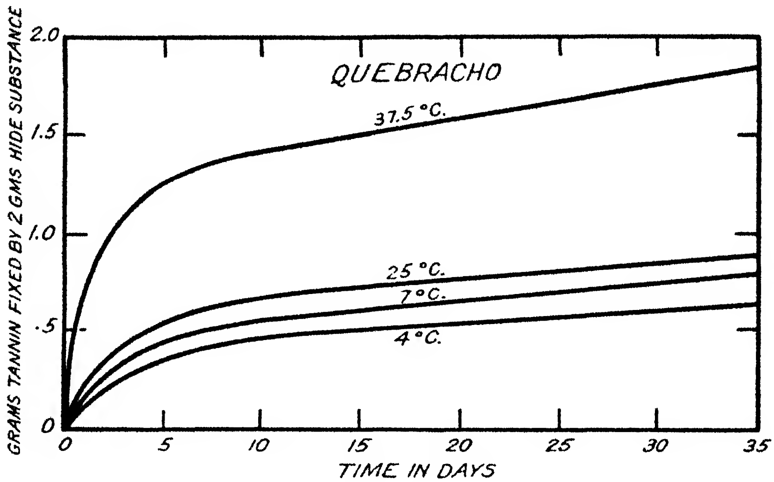


Figure 164

period of tanning was eight days at the temperatures noted. The percentages of combined tannin based on hide substance of the leathers were at: 10°, 28; 20°, 42; 30°, 51; and 40°, 62.

Page and Holland[75] have tanned steer hide with wattle-bark extract for six weeks at two temperatures, 15° and 35°, and at three pH values, 3.0, 5.0, and 8.0. Their results are shown in Table 244, where the percentage values shown are based on the hide substance of the leathers. It will be noted that

Table 244

	pH 3.0		pH 5.0		pH 8.0	
	15°	35°	15°	35°	15°	35°
Combined tannin (%)	36.8	47.7	32.4	45.8	33.6	43.1
Combined water-solubles (%)	41.0	35.0	38.5	31.4	40.0	27.1
	77.8	82.7	70.9	77.2	73.6	70.2

the combined tannin increases with rising temperature at each of the three pH values studies, whereas the combined water-solubles decrease. The value of all temperature effect studies would be enhanced by knowledge of dispersion changes of tannin solutions induced by temperature variation.

Effect of pH value on Tannin Fixation

In 1920, Atkin and Thompson[6] called attention to the wide variations in the pH values of solutions of different tanning materials, all of which were of

analytical concentration, or approximately 4 grams tannin per liter. The pH values ranged between 3.5 for chestnut wood extract to 4.3 for mimosa. The effect of varying the pH value of analytical solutions of various materials upon the amount of tannin shown by the official method of analysis was then studied by both Atkin[4] and Rogers.[92] Considerable variation in tannin content as a function of the pH value of the solutions was found. In the case of quebracho it was noted that, whereas the tannin content decreased and the insoluble matter increased with lowering of pH, the sum of tannin and insolubles remained a constant over a range of 2.5 and 6.5. But this constant value was not obtained for some of the other materials examined. Wilson and Kern[125] adjusted quebracho to varying pH values and then determined the

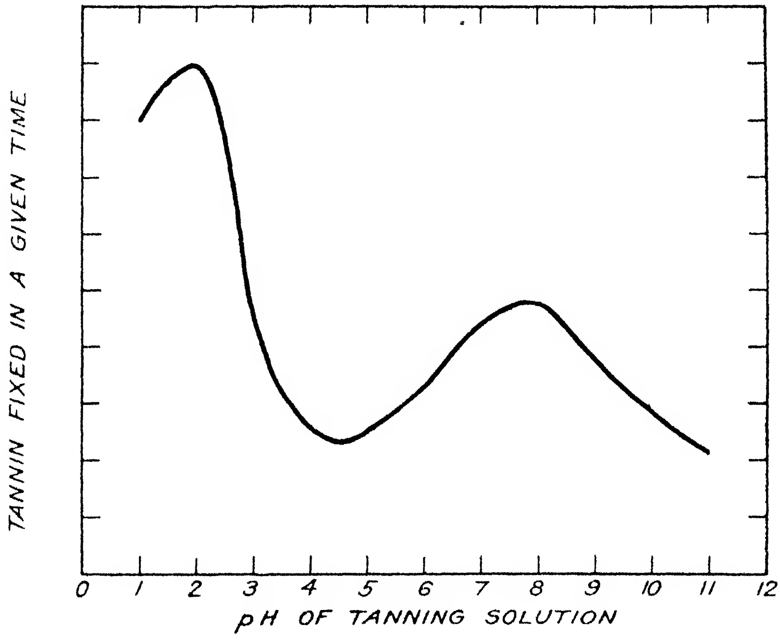


Figure 165

tannin by their method which, as has been noted, gave the more or less irreversibly combined tannin only. No variations of tannin values were found over the pH range 3.6 to 7.3; but it must be remembered that in this method the solutions are filtered before analysis and the insolubles are thus removed.

Thomas and Kelly[113] have published many experiments dealing with variation of tannin fixation by hide powder as a function of pH value. All their specimens were washed after tanning, and the fixed tannin values found were the irreversible fraction. The varying pH values employed were

obtained by adding the required amounts of hydrochloric acid or sodium hydroxide, as indicated by electrometric determination. Thomas[109] has summarized the results in a general curve, reproduced here as Figure 165. The significance of this curve will be discussed in the section dealing with the theory of vegetable tanning.

As noted above, the effect of pH variations shown in Figure 165 were obtained by the addition of hydrochloric acid to those tannin solutions whose pH was lowered. Since hydrochloric acid would not be present in normal tanning solutions, Thomas and Kelly[109] investigated the effect upon tannin fixation when a wide variety of acids was used to adjust tan liquor pH values. Very considerable differences in tannin fixation were found to result in the presence of various acids at the same tan liquor pH value. Thomas and Kelly suggested that the variation in tannin effect could be correlated with the dissociation constant of the added acid; in other words, the order of tannin fixation was found to be inversely proportional to the dissociation constant of the added acid, acetic acid showing the greatest effect and hydrochloric the least.

Page and Holland[78] have studied the effect upon tannin fixation by hide powder from wattle bark liquors of varying pH values. Their results are of particular interest, since they have considered the effect of pH on the various types of tannin fixation, as shown in Table 263 in the section dealing with reversibility of tannin fixation.

The experiments described above were performed with hide powder. Of possibly greater interest are those which follow, since they deal with hide itself.

Pawlowitsch[79] studied the behavior of pelt toward quebracho infusions of varying pH values, which were adjusted by addition of hydrochloric acid or sodium hydroxide. He noted that diffusion was greatest at pH 7.0-8.0, where tannin fixation was least. On the basis of these observations, he suggested a tanning process which consisted of placing hides in strong tan liquors of pH 6.0-8.0, and when they were struck through, of completing the tannage at pH 3.0-4.0, whereby maximum fixation could be obtained. Phillips[82] has recently been granted a patent for a tanning process based on this principle.

The effect of pH value on tannin diffusion and fixation in the case of steer hide was studied by Wilson.[134] Butt portions of the bated hide were suspended in tan liquors of increasing strength (0.5 to 6.0 per cent) for 42 days, a fresh liquor being given every two days. The pH values of the liquors were adjusted and continuously controlled by additions of lactic acid or sodium hydroxide. Two different tanning blends of equal parts of tannin of each material in the blend were employed. Blend (a) consisted of chestnut, quebracho, and cutch, blend (b) of chestnut, quebracho, and wattle. The results are shown in Table 245.

Table 245

pH Value	Parts Tannin Fixed per 100 parts H.S (a)	(b)	Days Required for Complete Hide Penetration (a)	(b)
3.0	56.8	45.0	42	42
3.5	50.6	36.3	24	21
4.0	43.0	37.8	24	17
4.5	42.2	37.2	19	18
5.0	37.3	31.9	19	17

Aabye and Rasmussen[1] have also studied the tanning behavior of mimosa-extract tan liquors of variable pH values, adjusted with different acids, with steer hide. Tannage lasted for 19 days in a series of liquors ranging from 5 to 50° barkometer and at pH values of 3.6 to 5.4. At the completion of tannage all specimens were drummed for 48 hours at 35° in a mimosa liquor of 145° barkometer which had been adjusted to pH 3.2 with the same acid which had been used for the pretannage. This retannage may have masked the actual tannin fixations at pH values higher than 3.2 in the pretannage. These authors reported, however, considerable differences in combined tannin with the tannages described as a function of the different acids used. Some of their results confirmed the contention of Thomas and Kelly that the order of tannin fixation was inversely proportional to the dissociation constant of the added acid; but the remainder of their experiments did not.

Effect of Concentration of Tannin Given

Our knowledge of this subject, which is of great importance to both theory and practice, is not very satisfactory. Thomas and Kelly[111] have studied the influence of the concentration of various tanning materials on the fixation of tannin by hide powder. Two grams of hide powder were treated with 100 ml of tannin solutions of increasing concentrations at various pH values and for different time periods. At completion of tannage, the tanned powders were washed free of soluble matters, and the combined tannin was determined. When these values were plotted against the concentrations given, a curve was obtained which showed a rapid initial rise in fixation; this usually occurred at a concentration of 15 to 20 grams tanning material solids per liter, after which the fixation curve abruptly descended and finally flattened out. When further and tremendous concentrations of material were employed, the flattened portion of the curve showed a slight rise in tannin fixation. Since the amount of the tanning materials given on hide substance basis ranged from 50 to enormous values of more than 1000 per cent, it is difficult to interpret the curves. Thomas[109] has suggested, however, that the sharp drop noted in the curve resulted from the hide powder being so heavily surface tanned by the strong solutions used that the tannin could not penetrate to the interior of the powder. He also suggested that increasing concentration had decreased the dispersion of the tannin, or that the value of the negative

electrical charge of the tannin particles was decreased as the tannin concentration of the solutions increased. He suggested that the second rise found in the fixation curve might result from the ability of the very highly concentrated solutions to overcome the case-hardening or other fixation-inhibitory qualities of the lower tannin concentrations.

Gallay[37] has studied the effect of increasing concentrations of pure tannic acid on tannin fixation. He employed as experimental material not only hide powder, but casein and gelatin. He did not, like Thomas, wash the tanned specimens and then determine combined tannin; by means of the Löwenthal oxidation method he determined the unabsorbed tannin left in the tanning solution. Deducting this value from that of the tannin given indicated the "tannin absorbed." When the tannin absorbed by either gelatin or casein was plotted as ordinate and the tannin unabsorbed as abscissa, a parabolic curve was obtained; and when the logarithms of these values were plotted, a straight line resulted. But when Gallay employed hide powder and tanned it with tannic acid concentrations of 25 to 50 per cent (on hide-substance basis) an entirely different type of fixation curve was obtained. That is, Gallay's hide powder results yielded a curve somewhat similar to those of Thomas and Kelly, when plotted in the same manner. Gallay's explanation of the type of curve he obtained differed from Thomas's interpretation of the same phenomena. Gallay felt that the shape of the curve was governed by the ability of the hide powder to swell as tanning proceeded. Gallay's hide-powder curve did not, however, show the second rise in fixation which Thomas and Kelly secured; this may be because the former did not employ more than 500 per cent tannic acid on hide substance, whereas the latter used up to 1625 per cent.

The most recent investigations of this subject are those of Chang and Doherty[20] and of Lollar,[59] who studied the fixation by hide powder of both tannic acid and a specially purified Chinese nut gall tannin. Like Gallay, they analyzed residual tannin solutions by the Löwenthal method and thus determined the "tannin absorbed" by the hide powder. They employed solutions ranging from 18 to 450 per cent tannin on hide substance basis. When the "tannin absorbed" values in their experiments were plotted against the unabsorbed, parabolic curves resulted. When the data were plotted according to Langmuir's method, straight lines were invariably obtained; they believed this indicated that vegetable tanning is of adsorptive nature. Their studies have been extended by Lollar, employing ordinary quebracho, and with essentially similar results and interpretations.

It is very difficult to attempt to evaluate or to compare the experimental results of these various workers, since totally different experimental methods are involved. Thomas and Kelly reported combined tannin only, whereas Gallay, Chang and Doherty and Lollar included three values in their "tannin

absorbed:" firmly combined tannin, that loosely combined, and that not combined at all but merely mechanically held. It is to be hoped, therefore, that this important subject will be reinvestigated, employing hide or skin itself rather than the unsatisfactory and inconclusive hide powder. Such studies should include both the purer tannins, such as tannic acid, and commercial tannins. Data as to changes in dispersion degree as a function of concentration of the solutions employed would no doubt be particularly illuminating. We would suggest that both combined tannin and combined water-solubles be determined, since "tannin absorbed" values are not particularly enlightening.

Rate of Tannin Fixation

The rate at which tannin is fixed by hide substance varies with the nature of the tanning material employed and the conditions under which it is used, as we have seen—with temperature, concentration, pH value, and other factors.

In 1919, Oscar Riethof[90] determined the rate of combination of steer hides with the tannin present in the blended liquors from a tannery employing a tanning process of 142 days. If we express as 100 the amount of tannin fixed at the end of the 142 days and relate thereto the tannin fixed for various periods during the process, the following figures are found: after 12 days, 32; 22 days, 38; 31 days, 55; 46 days, 64; 71 days, 78; 103 days, 86; and 142 days, 100. These figures are typical of what would be termed today a very long tanning process.

Page and Holland[75] tanned butt pieces of salted cow hide which were soaked, limed, unhaired, and bated, in wattle-bark extract at a pH value of 4.0. The liquors were gradually strengthened during the first three weeks and the specimens were then placed in liquors containing 100 grams solids per liter. Samples were removed at various time periods and were dried and analyzed. The results are shown in Table 246.

Table 246

Length of tannage	3 wks.	6 wks	6 mos.	1 yr.	2 yrs.
Combined tannin on H.S. (%)	18.0	35.5	40.4	43.6	49.0
Combined water-solubles on H.S. (%)	22.3	37.3	42.6	39.5	39.2
	40.3	72.8	83.0	83.1	88.2

It will be noted that the combined water-solubles tended to reach a constant value at the end of six months tannage but that the combined tannin continued to increase.

The same authors have also reported analyses of leather tanned with wattle-bark extract for tremendously long tanning periods. A flesh split

from a shoulder tanned for twenty years showed 87.6 per cent combined tannin and 54.8 per cent combined water-solubles, all on hide-substance basis, and a cow hide butt tanned for fifteen years showed 119.5 per cent and 37.8 per cent, respectively.

Stather and Lauffmann[100] determined the rate of tannin fixation from different materials, employing the corium of uncured cow hide—that is, hide substance from which the grain and flesh had been mechanically removed, and which was not subjected to the curing or liming processes. The corium was dried in a cool air current and was then cut into strips 15x2x1 mm. Twenty-five grams of dry corium were soaked at 15° and then tanned for a total of 64 days at the natural pH value of the tanning material employed. The strength of the tannin solution was gradually increased from 0.4 to 7.0 per cent. Specimens were removed at the intervals shown in Table 247, were washed free of soluble matter, and were then dried and analyzed. In expressing the results in the table, we have taken the value of combined tannin at the end of 64 days as 100 and have related thereto all the other values found for the various periods, for each of the materials studied. The right-hand column of the table shows the actual amount of combined tannin based on hide substance, at the end of 64 days' tanning.

Table 247

Material	Days Tanned							Combined Tannin after 64 days (%)
	1	2	4	8	16	32	64	
Ordinary quebracho	12	36	57	71	93	100	100	53.3
Sulfited quebracho	17	37	58	73	84	99	100	52.2
Valonia	16	32	53	70	85	98	100	45.6
Chestnut wood	27	56	74	90	96	100	100	46.2
Oak wood	22	36	49	62	76	88	100	41.8
Pine bark	14	33	52	68	77	88	100	30.5
Mimosa	14	33	51	75	89	98	100	51.9
Myrobalans	23	41	57	72	83	99	100	51.3
Sumac	27	44	62	77	88	99	100	51.3
Gambier	8	28	50	67	83	96	100	42.1

Merrill[63] tanned bated calf skin with oak-bark extract at a pH value of 4.4 and found at the end of 30 weeks that combination was still occurring at a considerable rate.

With the exception of the data of Riethof, all the above data were obtained with single materials. Further knowledge of the tanning rates of typical tannery blends should be of value.

Weight and Thickness Changes of Hides and Skins in Vegetable Tan Liquors

When hides or skins are placed in contact with solutions of vegetable tannins, they may change in thickness, in weight, or in both respects. Such

changes are a function of many controlling factors, the regulation of which constitutes an important part of the tanning process. The first problem which arose in the control of weight or thickness changes was a dependable method for their measurement.

In 1920, McLaughlin and Porter[60] made an extended study of the manner in which limed hide, which had not been delimed or bated, changed in weight when placed in weak tan liquors of varying constitution. To these liquors were added such typical nontannin substances as gallic acid and pyrogallol, such organic acids as lactic and acetic, and such typical salts as calcium lactate and calcium acetate. The limed hide pieces were weighed before and after immersion for varying time periods in the several liquors, and the weight changes were noted. These workers did not measure the pH values of the liquors of their experiments, and the lack of such information limits the interpretation of their results. The experiments did, however, show that the character of the nontannins of a tan liquor had a very important bearing upon the weight behavior of the hide with which they were in contact.

Wilson and Gallun[130] successfully solved the problem of measuring thickness changes of hide or skin by the employment of a Randall and Stickney gauge, with which they quantitatively determined the resistance to compression of skin specimens. They employed pieces of calf skin butt which were limed, unhaired, and delimed. The specimens were placed in liquors made with oak-bark extract, to which had been added increasing amounts of lactic acid; the effect of adding increasing amounts of sodium chloride to a liquor containing a constant amount of lactic acid was also studied. Measurements of compression resistance of the skin before and after the treatments described showed the plumping effect of added lactic acid and the depression of acid-plumping as a function of added sodium chloride. Page and Gilman[73] have employed this method in measuring the plumping effect of various acids added to wattle-bark liquors. They used cow hide which had been limed, unhaired, and washed, but not bated. When the plumping values they obtained were plotted against the pH values of the residual liquors, the curves showed identical plumping values of added hydrochloric and lactic acids, and about one-half of such values when the dibasic sulfuric acid was employed. R. E. Porter[84] has also successfully employed the Wilson and Gallum method, using kip grain and cow hide and oak-bark liquors to which varying proportions of lactic acid and of sodium chloride were added. Porter has also studied the thickness changes of cow hide when immersed in aqueous solutions of $0.1M$ lactic acid and when the acid solution was made $0.1M$ with various neutral salts. He found that the changes in plumpness induced by the salt/acid solutions could be fairly well correlated with the well known Hofmeister series of salt effects; but no such relation was found in the case of weight changes of the treated hide.

Effect of Drying and of Aging Leather on Tannin Fixation

Tanners of vegetable leather have long known that the amount of tannin combined with hide substance is increased both by drying the leather and by storing the leather after drying. This increase in combined tannin may reach very considerable values, as illustrated by the experiments of Wilson and Kern[132] given in Table 248. They tanned hide powder with the various tanning materials shown, washed the specimens free of all soluble matter, and then determined the amount of combined tannin contained. Duplicate portions were dried after tanning *without washing*; one set was kept at room temperature for thirty days and another portion one year, and at the end of each storage period they were washed free of soluble matter, and the combined tannin was determined. In Table 248 we have expressed the per cent combined tannin of the undried specimens as 100 throughout and have related thereto the dried and stored specimen values.

Table 248

Material	Undried Leather Washed Immediately	Dried Leather Washed after 30 days' storage	Dried Leather Washed after 1 year storage
Quebracho	100	114	115
Gambier	100	135	167
Gambier-quebracho mixture	100	118	125
Chestnut wood	100	129	131
Hemlock bark	100	106	109
Chestnut wood-hemlock bark mixture	100	109	113
Oak bark	100	112	131
Larch bark	100	117	166
Sumac	100	110	110
Wattle bark	100	107	110

We note that the combined tannin values are affected by the drying and aging of the tanned hide powder and that these increases vary considerably with the type of tanning material employed. Since these experiments were made with hide powder entirely, similar studies with hide pieces would be instructive, and they should include other tanning materials, such as mangrove (cutch), valonia, myrobalans, etc. The behavior in this respect of the various blends which are typical of modern practice would also be helpful.

Acidity of Tan Liquors and their pH Value

Tanners have long known that the acidity of tan liquors has a very important bearing upon the amount of tannin fixed by hide substance, the rate of such fixation, and the color, weight yield, and character of the leather produced. Before the advent of the leather chemist, the control of tan liquor acidity was an important part of the tanner's art, and his ability to regulate acidity conditions was often amazing. But with the introduction of a greater variety of tanning materials in tanning blends and with the reduction of

tanning time and hence less natural fermentation, the problem became much more complicated. The leather chemist has fortunately been able to supply adequate methods of acidity estimation and control.

The older analytical procedure consisted essentially of detannizing a tan liquor by means of gelatin and then titrating the decolorized nontannin fraction with alkali in the presence of a suitable indicator. The acid was then calculated and reported as "per cent acid as acetic." This procedure obviously does not differentiate between the various titratable organic acids present, nor does it tell anything regarding their dissociation. Two tan liquors with identical titration values may behave in an entirely different manner toward hide substance, as McLaughlin and Porter[60] have shown.

A tan liquor may contain a variety of organic acids, the principal ones probably being lactic and acetic, together with smaller amounts of formic, butyric, and possibly others. It will also contain gallic acid, the amount of which will vary with the nature of the tanning material constituting the liquor and with the degree to which the hydrolyzable tannins have been decomposed by mold ferments such as tannase. Cameron and McLaughlin[18] have devised methods for differentiating and accurately estimating acetic, lactic, and gallic acids present in a tan liquor. The acid values shown in Table 235 were determined by these methods. Highberger and Youel[49] have suggested a modified method of lactic acid determination which is more rapid than that of Cameron and McLaughlin and probably equally satisfactory. Tanning experience indicates that the presence of any excess of gallic acid in a tan liquor tends to give flat leather, the weight yield of which is often low.

In 1911 J. T. Wood[136] introduced the electrometric estimation of the concentration or activity of hydrogen ions present in tan liquors, although nearly a decade was to pass before leather chemists took advantage of this invaluable tool.

Wood and his associates, Sand and Law[137] employed the hydrogen electrode in examining and titrating vegetable tan liquors. Cameron and McLaughlin[18] extended this work, using both the hydrogen and glass electrodes in the titration of typical tannery liquors. Alkali was added to the tan liquor and the pH value was determined after each addition. When the results were plotted, with alkali additions as abscissa and the pH value found as ordinate, curves very similar to those of Wood, Sand, and Law were obtained: the curves showed no vertical section which would correspond to a complete neutralization of acids present; in other words, vegetable tan liquors were found to be buffer systems. Since this buffering capacity varied greatly, Pleass[83] suggested that the reserve or un-ionized fraction of acid in a tan liquor be termed its "buffer index," which she defined as the number of ml of normal hydrochloric acid or sodium hydroxide required to be added to 100 ml of a 20° barkometer liquor to alter its pH value by one unit.

Summing up: The electrometric determination of the pH value of a vegetable tan liquor gives us one of the most informative of figures in studying or controlling the tanning process, since it designates the active acidity of the system. With the advent of the glass electrode, this value may be easily and quickly obtained. But it must be kept in mind that the determination, as such, tells us nothing as to the nature of the acidic bodies which yielded the hydrogen ions the concentration or activity of which is determined. With these thoughts in mind, let us proceed to specific applications and effects of the pH value.

Effect of pH Value on Liquor and Leather Color

The color of both tan liquors and the leathers made therefrom is influenced by the pH value of the tan liquor. This does not mean, however, that the color of the finished leather will necessarily be that of the liquor in which it was tanned. Because of this, and since the purchaser of tanning materials is greatly concerned with the color to be imparted to his leather, many efforts have been made to determine their color quality. One method of color evaluation is to tan sheep skin skivers with a dilute solution of the tanning material and to note the color of the tanned skiver after washing and drying. This method has not proved very satisfactory, because it does not duplicate tannery conditions. Another method involves the comparison of the color of a tannin solution with standard colored glasses.

Procter[87] and Blackadder[11] have independently suggested methods based upon the red, yellow, green, and blue regions of the spectrum. Blackadder measured the amount of light passed by the tan solution under examination and under standard conditions. Separate measurements were made in the following spectrum divisions: Red wave length 600 to 700, yellow 550 to 600, green 510 to 550, and blue 400 to 510. Employing Blackadder's method, Oberfell[71] has reported extensive color measurements of chestnut, quebracho, oak, and hemlock extracts.

Effect of pH Value on Insolubles

Thomas and Foster[110] added increasing amounts of sulfuric acid to solutions of various tanning materials containing 40 grams solids per liter. After adding the acid, the solutions were allowed to stand 30 minutes and were then centrifuged for five minutes at 1000 times gravity, and the volume of the precipitate was measured. No pH values were determined. The precipitate volume did not vary greatly for the different tanning materials. Numerous curves were given showing the effect of the addition of various other acids and of different salts upon precipitation. But all these results were reported as the "volume of precipitate." The volume of the precipitate did not necessarily bear any direct relation to the weight of the precipitate, nor

to the actual percentage which the precipitated matter was of the total solids of the tan liquor.

Wilson and Kern[129] adjusted the pH values of ordinary quebracho solutions containing 4.0 grams tannin per liter with hydrochloric and sulfuric acids, and with sodium and calcium hydroxides, respectively. Determined electrometrically, the pH range was from 2.0 to 11.0. The adjusted solutions were then analyzed according to the official method, and the insoluble matter was determined. The results are shown in Figure 166. The pH value of the original quebracho solution was 4.6. It will be noted that as the pH value was lowered by either acid, the insolubles increased, sulfuric acid being slightly more effective in this respect. Addition of sodium hydroxide

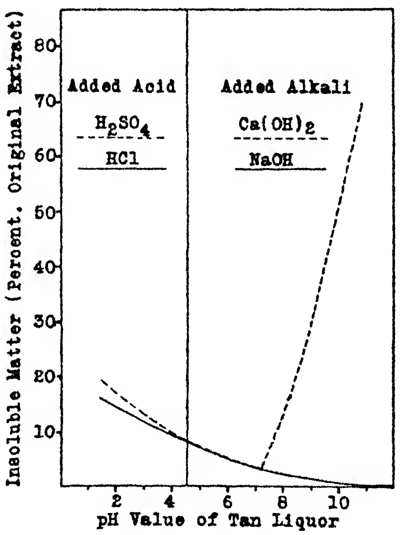


Figure 166. Effect of pH value on per cent of insoluble matter in solution of quebracho extract.

decreased the insolubles percentage at all pH values, whereas addition of calcium hydroxide caused a tremendous increase after pH 7.0 was passed. As Wilson and Kern pointed out, the data pertaining to the effect of calcium hydroxide should be of great importance in considering the character of water used in leaching or in dissolving tanning materials.

Gerngross and Herfeld[39] have extensively studied the behavior of various materials (gambier, cutch, quebracho, wattle, chestnut wood, oak wood, mangrove, myrobalans, valonia, sumac, pine, maletto, oak bark, algarobilla, and divi-divi) toward the addition of various acids to 0.4 per cent tannin solutions. Mineral acids were slowly added to these materials and the pH value at which precipitation occurred was noted. It was found that the

condensed tannins, with the exception of wattle (mimosa), had characteristic precipitation points between pH 7.0 and 1.0; this did not apply to the hydrolyzable tannins. There was no appreciable difference in the effect of sulfuric and hydrochloric acids. Organic acids produced an effect different from that of inorganic, in that at higher concentrations the organic acids had a peptizing action on precipitated substances. When solutions of the condensed type of tannin were aged, there was a continual increase in the acid-precipitable fraction; but this was not true in the case of the hydrolyzable tannins. These authors have also determined the quantitative effect of lowering the pH value of various materials by the addition of hydrochloric acid. The percentage of insolubles in liquid ordinary quebracho at its natural pH value of 4.95 was 2.4 and 8.5 when the pH value was reduced to 1.6; solid ordinary quebracho 7.9 at pH 4.6 and 15.2 at pH 1.9; oak wood 1.0 at pH 3.7 and 8.0 at pH 1.0; and chestnut wood extract 0.8 at pH 4.05 and 5.6 at pH 1.6. These authors suggested that acid precipitation of the tannins involved both their electrical discharge and a reduction in their degree of hydration.

The reaction of various tannins to changes in pH value induced by the addition of different types of acids or alkalies is important. It is also important to know the effect of added salts. But such information appears to be of appreciable value only when detailed analyses of the materials before and after treatment are given. The value of any future experimental studies in this connection would be enhanced by the employment of stronger liquors, possibly of 30° barkometer concentration, rather than the very weak concentration of analytical strength. Quantitative studies of changes in dispersion of the treated liquors would also be of value.

Fermentation of Tan Liquors

As has become evident in preceding sections, the amount of organic acids in a tan liquor, and the liquor's pH value, are of importance. The amount and kind of acids present, and their dissociation, vary with the kind of tanning materials used and with the nature of the tanning process employed. When tanning was performed entirely with materials rich in fermentable sugars, and when the tanning process was very long, the requisite acids were derived from the tanning materials themselves by means of natural fermentation, which thus produced "sour" liquors. Regulating this fermentation was an important part of the tanner's art, and he often achieved amazing success without the aid of chemistry and before "pH" values were known. The modern trend toward much shorter tanning processes and the employment of less fermentable tanning materials has lessened the importance of the fermentation of tan liquors. The pH values of tan liquors in modern tanneries are closely regulated, since the advent of the glass electrode has made this easily possible. And where pH adjustment is needed, it is often accomplished

by the addition of the requisite amount of organic acid, usually lactic, to the tan liquor. In some cases the desired pH value of "tail" or weak and nearly exhausted liquors (those into which the hide is first introduced) is obtained by addition of the cheaper sulfuric acid. The subject of tan-liquor fermentation is still one of importance and interest, however.

It was first adequately studied by Andreasch,[2] who published a series of articles about it in 1895-96. The next detailed investigation was that of Blank,[12] in 1935. He studied the fermentation of oak-bark extract and its formation of lactic acid and showed that the microörganisms it contained could be divided into two major groups: those which produced lactic acid through fermentation of its sugars, and those which destroyed lactic acid. Increasing tannin concentration retarded the activity of both groups, while absence of atmospheric oxygen usually retarded the activity of the acid-destroying group, but did not affect the acid-producing group.

The studies of Blank have been greatly extended by Doelger[29] and also by Doherty.[30] Doelger found that the lactic acid-producing organism most frequently present in tannery rocker liquor was *streptococcus lacticus*, with relatively few *lactobacilli*, and that the most frequent acetic acid-producing organism present in tan liquors was *acetobacter aceti*. All these organisms grew best in a temperature range of 25° to 30°. He found no acid production at 17°. The growth of all these organisms was governed by the reaction of the tan liquor, and he found that the production of lactic acid was inhibited in rocker liquors when the pH value was less than 4.0.

Doherty isolated *lactobacilli* from tannery rocker liquors. He then inoculated sterile solutions of different tanning materials with this organism and studied the production of lactic acid, finding that the various materials vary greatly in their fermentability by these organisms. All his studies were conducted at 28°, and he stated that if this temperature was reduced to 18°, the fermentation was greatly retarded. No destruction of tannin as a result of bacterial fermentation was found.

Seltzer and Marshall[96] allowed 35° barkometer liquors made from various tanning materials to stand 12 weeks, with and without exposure to atmospheric oxygen. The liquors were analyzed periodically for tannin and for titratable acids. Very considerable decreases in tannin content were noted in the case of some materials and very little in others. For example, at the end of eight weeks' exposure the myrobalans liquor had lost 54 per cent of its original tannin but showed a slight decrease in titratable acid, whereas mangrove had lost only 1.5 per cent of tannin, but its titratable acid value had increased six-fold. These authors ascribed the tannin losses to fermentation but did not offer any evidence of the nature of such fermentation, or whether their liquors contained hydrolyzing molds.

Effect of Adding Nontannins to Tannins

Since the nontannins present in a tan liquor are known to affect greatly the tanning behavior of the liquor, Thomas and Kelly[113] have investigated the effect of adding the typical nontannin materials, gallic acid and pyrogallol, to solutions of wattle-bark extract and of gallotannic acid. They studied the fixation of tannin by hide powder from such treated liquors. Their results are somewhat confusing, since the degree of tannin fixation was decreased in some cases by the added nontannins and increased in others.

Stather and Herfeld[99] have investigated the effect of increasing the concentration of the normal nontannins present in tan liquors made from different materials upon their capacity of tannin fixation. The nontannins were obtained by detannizing a 5.0 per cent solution of the material with gelatin/salt solution and then with hide powder. The nontannins were then concentrated *in vacuo* at 45-50° and were added to stock solutions of the parent material. These additions were such that three experimental liquors were obtained containing (1) the normal proportion of nontannins, (2) twice the normal, and (3) four times the normal; all three solutions contained the same amount of tannin. These liquors were then used to tan pieces of delimed calf skin butt. Tannage was performed in rotating glass drums for 35 days, the liquor being gradually strengthened during the first 15 days. At the completion of tannage the leather was superficially washed with water and then dried and analyzed. The results are given in Table 249. The table shows the amount of fixed tannin present in the various leathers after long washing, and we have expressed the tannin fixed from the normal liquor as 100 per cent in the case of each material, relating thereto the fixations with added nontannins.

Table 249

	Pine bark	Valonia	Ordinary quebracho	Oak wood	Sumac
Fixed tannin, normal liquor (%)	100	100	100	100	100
Fixed tannin, double nontannins (%)	95	96	97	94	91
Fixed tannins, quadruple nontannins (%)	88	94	95	91	89

The results shown in the table indicate a definite effect by nontannins on tannin fixation, but the reason for this is not clear. Knowledge of the changes brought about in the degree of dispersion of the tannin bodies as a function of added nontannins would no doubt be of value.

Oxygen Effects in Vegetable Tanning

It is often stated that atmospheric oxygen has important effects upon vegetable tan liquors and on the color of the leather tanned therein, as well as in the drying of leather after tanning.

We have already noted on page 569 the fact that if oxygen is passed

through a solution of nontannins, or if the solution is merely exposed to air for long periods, some sort of change is brought about in the solution whereby it gives a tannin reaction; and we have noted the same result when a solution of gallic acid is oxidized. Merry[65] has, however, been unable to find any oxygen take-up in these transformations.

Wilson and Kern[129] made up 1.0 per cent tannin solutions of both quebracho and gambier. These solutions were brought to a pH value of 2.5 by the addition of phosphoric acid; sodium hydroxide was then added to give a series of each material ranging from pH 3.0 to 12.0. The gambier solutions varied in color from a light straw color at pH 3.0 to a deep red at 12.0; the quebracho solutions showed a similar color trend, except that the solutions of the lower pH values had a touch of violet. These color variations disappeared in both series, however, when all the solutions were brought to a pH value of 3.0, providing they had not been exposed to air. But when they had been exposed to air and were then brought to pH 3.0, they showed great differences in color; those solutions which had been exposed to air at the highest pH values showed dark color.

By means of Barcroft's blood gas manometer, Jany[56] determined the actual amounts of oxygen absorbed by different materials and at different pH values, when 2 ml of solution were shaken with oxygen for one hour at 37.5°. The results are expressed in Table 250 as the percentage of oxygen absorbed per 100 grams tannin or other material examined.

Table 250

Material	pH Value				
	2 0	4 5	5 3	9 2	12 0
Blended tannery liquor	0.00	0.00	0.04	2.14	6.830
Pure tannin	...	0.00	..	0.51	5.630
Pyrogallol	..	0.00	..	3 91	6.860
Phenol	.	0.00	.	0.00	0.013
Glucose	..	0.00	..	0.00	0.098

Merry[65] has also extensively studied the oxidation of tannins by means of the Barcroft manometer. He determined the amount of oxygen absorbed by various commercial tannins as a function of time and at a temperature of 18-20°, employing 1.0 grams of solid extract dissolved in 3.0 ml of distilled water. At the end of 75 hours' treatment the materials had absorbed from 0.28 to 0.03 per cent oxygen per gram of solid extract. The order of decreasing oxygen absorption was: ordinary quebracho, sulfited quebracho, wattle, mangrove, American chestnut wood, and myrobalans. The rate of absorption increased with increasing temperature, with increasing pH values, with the presence of mold growth, and with the presence of copper or iron compounds. The absorption was decreased by the lowering of pH values by addition of acid to the liquors.

Referring to Table 263 (p. 607) it will be noted that Page and Holland found the degree of tannage of hide powder by wattle-bark extract at pH 8.5 to be very much greater in the presence of air than in its absence; but it will be recalled that this phenomenon was not found when hide was employed instead of hide powder. Page and Holland suggested that this was due to the inability of the larger oxidized tannin molecules to penetrate the compact structure.

In attempting to sum up this subject, it would appear that under manufacturing conditions of vegetable tanning, and at the pH values of tannery liquors, the effect of atmospheric oxygen cannot be very great. On the other hand, there can be no doubt that atmospheric oxygen may have marked effects upon the color of leather during drying.

Tanning with Tannin Dissolved in Organic Solvents

While vegetable tanning is invariably performed with aqueous solutions it is important from a theoretical standpoint to know to what extent the reaction may occur when tannin is dissolved in other solvents. Chambard and Mezey[19] have studied the fixation by anhydrous hide powder of tannin dissolved in absolute ethyl alcohol. They found negligible amounts fixed. This finding was confirmed by Gallay.[37]

Stather, Lauffmann, and Bau Miau[101] tanned hide powder with various materials dissolved in different solvents and then washed the *undried* leathers with the corresponding pure solvent. The washed leathers were then dried and weighed and the combined tannin determined. We have expressed their results in Table 251 as the percentage of combined tannin as related to the fixation in aqueous solution, which is taken is each case as 100 per cent.

Table 251

Tanning material	Water	Methyl alcohol	Ethyl alcohol	½ Water ½ Ethyl alcohol	Acetone
Ordinary quebracho	100.0	6.2	1.0	25.7	1.0
Sulfited quebracho	100 0	6.5	1.6	44.4	. .
Mimosa	100.0	5.9	1.2	32.9	. . .
Chestnut wood	100.0	6.7	2.0	44 4	0.0
Sumac	100.0	12.5	2.3	29 2	.
Valonia	100.0	10.6		.	. .
Myrobalans	100.0	14.2	3.7	36.0	

Page[77] shook purified hide powder with an excess of purified wattle-bark tannin dissolved in various solvents for 48 hours, washed the tanned powder with the pure solvent, dried the leather, and analyzed it for combined tannin. He found no fixation to occur in the case of either ethyl acetate or acetone, but 99 parts tannin per 100 hide substance were fixed from tannin dissolved in acetic acid and 15 from that dissolved in formic acid.

Chang and Doherty[20] have studied the tannin take-up by hide powder

from 75 per cent ethyl alcohol solutions. This was done by analyzing the residual tan liquors for tannin by the Löwenthal oxidation method and calculating therefrom the amount of tannin absorbed by the hide powder. This method does not of course permit the differentiation of combined and uncombined tannin, as in the case of the washing method. However, it indicated a tannin take-up of only about 10 per cent on hide substance, regardless of the concentration of tannin given.

Chambard and Mezey, as well as Stather, Lauffmann, and Bau Miau, believed the negligible fixations from ethyl alcohol and other solvents was due to lack of ionization of both hide substance and dissolved tannin. On the other hand, Page pointed out that fixation occurred from solutions of water, acetic acid, and formic acid, and that swelling of the hide substance occurred in each of these solutions, whereas it did not occur in acetone or ethyl acetate. He suggested that vegetable tannage may possibly be governed by the ability of the hide substance to swell, since otherwise the tannin molecules may be unable to penetrate between the protein chains.

Roddy[91] has extensively studied the tanning action of vegetable tannins dissolved in various organic solvents. He finds that no actual tannage occurs when anhydrous collagen is treated with tannin dissolved in anhydrous acetone. Tannin fixation does occur, however, if water is present in the system; fixation is roughly proportional to the amount of water present. Roddy placed bated steer hide in an acetone solution of 40 parts ordinary quebracho extract per 100 parts acetone, and found that such solution completely penetrated the hide in 48 hours. The hide was then removed and was placed in cool water for 24 hours, during which period tannage took place. The leather was then dried and analyzed and showed 54 per cent tannin fixed. It will be noted that the acetone process just described differs from the ordinary tanning procedure; that is, the hide is first impregnated with tannin, and tannin is then fixed by the action of water. Roddy lists a number of organic solvents which may be used in his process but recommends those solvents, such as acetone or alcohol, which may be easily and completely removed by evaporation from the leather.

Our understanding of the phenomena of the marked differences in tannin fixation from various solvents would be greatly enhanced by data of the dissociation constants of different tannins dissolved in various solvents. No such values are to be found in the literature, but the example of the dissociation constants determined by Goldschmidt and Aas[40] for salicylic acid dissolved in various solvents may be considered as possibly indicative of differences to be found with the tannins. These workers reported the following dissociation constants for salicylic acid: water, 1.02×10^{-3}; methyl alcohol, 1.3×10^{-8}; ethyl alcohol, 2.2×10^{-9}. We would add that particle-size studies of tannins dissolved in different solvents would be helpful.

Effect of Pretannage with Chromium Compounds upon Tannin Fixation

This subject is important from a theoretical standpoint and because of its bearing on the production of the so-called "chrome retan" leathers.

In 1908, Wood[135] measured the tannin-combining capacity of chromed gelatin and found it to fix as much tannin as did unchromed gelatin; from this he concluded that chrome and tannin combine with different protein groups. But the experimental procedures known at that time were not such that these findings could be regarded as conclusive.

In 1927, Gustavson[45] made an extensive investigation of the subject. He tanned hide powder with solutions of the chromium compounds noted below, from which were fixed the amounts of Cr_2O_3 shown in Table 252. The chrome-tanned hide powder was washed free of uncombined chrome with distilled water, and the excess moisture was removed by suction filter until the moisture content of the chromed powder was about 70 per cent. These specimens, together with unchromed powder of similar moisture content, were then tanned for 48 hours at room temperature with hemlock bark extract or with tannic acid. The specimens were then washed free of soluble matter and were dried and analyzed. The results are shown in Table 252.

Table 252

	Control	Pretanned with 37% Basic Chromium Sulfate	Pretanned with 30% Basic Chromium Chloride	Pretanned with Sodium Oxalato Chromate
Hemlock bark extract, pH 4 0, 45 gms solids per l				
% Fixed tannin on H. S. basis	58.4	71.8	74.9	52.3
% Fixed Cr_2O_3 on H. S. basis	...	10.6	5.0	6.0
Tannic acid, pH 3 1, 80 gms solids per l.				
% Fixed tannin on H. S. basis	94 3	109.8	126.6	89.6
% Fixed Cr_2O_3 on H. S. basis	...	8.0	4 6	3.6

Commenting on these results, Gustavson stated that the fixation of tannin by hide powder tanned with either the cationic chrome compounds (chromium sulfate or chloride) or the anionic (sodium oxalato chromiate) is independent of the pH values of the tannin solutions employed. He considered the increased tannin fixation of the cationic chrome-tanned hide powder to be due to the chrome having combined with skin carboxyl groups which, in turn, activated basic protein groups and made them available for combination with tannin. He assumed that the lowered tannin fixation by the anionic chrome-tanned hide powder was due to the fact that both negatively charged chromium and negatively charged tannin were competing for the basic protein groups.

Thomas and Kelly[116] have reversed the conditions of Gustavson's experiments. That is, they first tanned hide powder with wattle-bark extract, washed out all soluble matter, and then tanned the powder with basic chrome sulfate; they found very much less fixed chrome than in the case of the control, which was not vegetable pretanned.

Otin and Alexa[72] have repeated and confirmed Gustavson's experiments, employing 33 per cent basic chrome alum for pretanning and quebracho for subsequent tanning, finding increased tannin fixation. Like Gustavson, they attributed this result to an activation of protein basic groups.

Page and Holland[76] seem to be the only workers who have employed hide, rather than hide powder, in studying this subject. They tanned hide pieces with 33 per cent basic chrome sulfate so as to attain a wide range of fixed chrome; these specimens were washed until "almost free from sulfates" and were then tanned for six weeks in wattle-bark extract; after this they were drained (not washed), air-dried, and analyzed. The results are given in Table 253.

Table 253

Fixed Cr_2O_3 on H S (%)	Combined tannin on H S (%) (a)	Combined water-solubles on H S (%) (b)	(a) + (b) (%)
0.0	33.1	44.9	78.0
3 9	49.1	42.2	91.3
4.5	47.7	35.1	82.8
6.3	60.1	32.4	92.5
6.7	61.4	28.7	90.1
7.3	69.6	25.5	95.1

It will be noted that the combined tannin increased with increasing chrome content, while combined water-solubles decreased. Commenting upon their results, Page and Holland made the pertinent suggestion that while chrome pretanning may not necessarily increase the essential ability of hide substance to fix tannin, it may increase the usually slow *rate* of tannin fixation.

As will be recognized from the foregoing, this subject is in a confused state. If fixed chrome combines with the carboxyl or acidic groups of hide substance and thus activates the amino or basic groups, making them available for increased tannin fixation, it follows *a priori* that pretannage with tannin should activate carboxyl groups and thus increase cationic chrome fixation. Thomas and Kelly showed that just the opposite happens. The subject is, however, of real importance and should be pursued further. Future experiments should be made with hide pieces and not with hide powder; and where chrome pretanned specimens are employed, they should be washed completely free of acid radical, both protein- and chrome-bound, since the presence of such radicals needlessly confuses experimental results and their interpreta-

tion. We have found that complete acid radical removal from chrome leather requires at least two weeks washing in running water.

Stiasny[106] has pointed out that the possible effect of pretannages upon the active surface of hide substance must be considered in attempting to understand combination tannages.

Effect of Pretannage with Formaldehyde on Vegetable Tannin Fixation

Gerngross and Roser[38] tanned hide powder with formaldehyde and then retanned it with tannic acid. They found that less tannin was fixed by the pretanned hide powder than by the control, and that the decrease of tannin fixation was a function of the amount of formaldehyde fixed before vegetable tanning. In a later study they extended their experiments to include oakwood extract and stated that whereas 19.77 per cent tannin was fixed by regular hide powder, that which was "lightly tanned with formaldehyde" showed 18.65 per cent and that "strongly tanned with formaldehyde" 15.02 per cent.

Chang and Doherty[20] have also studied the effect of formaldehyde pretannage of hide powder upon its ability to fix tannin. They tanned 1.0 gram hide powder in 100 ml of a solution containing 25 ml of molar NaOH, 10 ml of molar phosphoric acid, and 2.5 ml of formaldehyde (40 per cent) for 24 hours at room temperature with occasional shaking. The excess formaldehyde was then drained off, and the hide powder was washed several times with $0.2N$ Na_2SO_3, according to the method of Salcedo and Highberger. The excess of Na_2SO_3 was then washed out with distilled water. Excess water was squeezed out, and the formaldehyde-tanned powder was then placed in $0.1M$ phosphate buffer at the desired pH value for 24 hours. No statement of the amount of fixed formaldehyde is given. The formaldehyde-tanned powder so prepared was then placed in buffered solutions of different pH values, containing increasing concentrations of tannic acid and of purified Chinese nut-gall tannin; after this it was tanned for 24 hours at 21° with constant agitation. At the end of the tanning period the unabsorbed tannin was determined by the Löwenthal method, and the difference between this value and that of tannin given was expressed as "tannin taken up." The curves of such values indicated: when tannic acid was employed at pH 3.60 and up to 40 per cent tannic acid "taken up," there was essentially no difference between straight hide powder and the formaldehyde tanned; but in the higher tannin concentrations given, very much less tannin was taken up by the formaldehyde-tanned powder; where the take-up of tannic acid at pH 5.60 by the straight powder was compared with that of the formaldehyde-tanned at pH 5.80, the values of take-up of the two were essentially the same; and where the purified tannin was employed, there was practically no difference in take-up of the two powders at pH 5.00.

All the above values were obtained by the Löwenthal method; the "take-up" values secured therefrom do not permit differentiation between tannin firmly fixed, that loosely fixed, and that not fixed at all. It is therefore difficult to decide just what influence formaldehyde pretannage has had. These studies should be repeated, using skin itself, and the excess formaldehyde present after pretannage should be removed by hydraulic pressing. Values for actual tannin fixation should be secured, employing a single tannin concentration but varying, and accurately determining, the amount of formaldehyde fixed in the pretannage. In the event of actual decrease of fixed tannin as a result of formaldehyde pretannage, an effort should be made to determine whether such decrease is a function of removal of protein basic groups through their reaction with formaldehyde, whether the formaldehyde has changed the reactive surface of the hide substance, or whether it prevents swelling of the skin during vegetable tanning and the inward diffusion on the tannin aggregates.

The effect of formaldehyde pretannage upon the thermolability of vegetable-tanned leather is discussed later in this chapter.

Effect of Pretannage with Quinone upon Tannin Fixation

Since it had been suggested that both quinone and tannin combine with the same basic protein groups, Thomas and Kelly[115] logically assumed that quinone-tanned hide powder should show a lowered tannin fixation capacity. To demonstrate this they employed quinone-tanned hide powder containing 78.3 per cent fixed quinone on hide substance basis and tanned 2.0 grams of it with 100 ml of a solution containing 4.0 grams of tannic acid at various pH values for 24 hours. The thus doubly tanned powders were then washed free of all soluble matter with distilled water, and the fixed tannin was determined. The percentages of fixed tannin on hide substance basis are as shown in Table 254.

Table 254

pH of Solution	Tannin Fixed on H S Basis (%)	pH of Solution	Tannin Fixed on H S Basis (%)
1	3.4	7	5.9
2	2.6	8	8 9
3	3.5	9	− 8.7
4	3.5	10	− 27 8
5	4.8	11	− 31.9
6	4.4	12	− 40.7

It will be noted that only inconsequential amounts of tannin were fixed up to a pH value of 8.0. The weight losses at higher alkalinities were found by Thomas and Kelly to be due to the reversal of the fixed quinone at these alkalinities. They considered their data to be proof that quinone and tannin are actually fixed by the same protein groups.

Influence of Added Syntans on Tannin Fixation

The chemistry of that group of substances included under the term "syntans" has been dealt with in Chapter 21. There are many different syntans, and their effect upon tannin fixation may vary greatly with their composition. When they are added to a vegetable tan liquor, they usually, but not always, decrease the amount of insoluble matter present, they tend to increase the degree of dispersion of the vegetable tannin material, and they may increase or decrease the amount of tannin fixed by hide substance. These subjects have been dealt with by Wilson,[132] by Thomas and Kelly,[113] by Kohn, Breedis, and Crede,[57] by Stiasny and Orth,[104] by Stather and Löchner,[102] and by Berkman and Babun.[10]

Of possibly greater importance than the effect of syntan additions to the tan liquor itself is its effect upon the hide or skin which is placed in such an admixture. Bergmann, Munz, and Seligsberger[8] have shown that syntans cause a drastic alteration in the diffusion coefficient of limed and subsequently delimed hide. They did this by forcing through the hide various solutions of syntans under pressure, and they compared their speed of penetration with that of water, which was taken in each case as 100. Thus the penetration speed of the syntan Neradol ND when diluted 100 times was 140, when diluted 50 times, 330, and when diluted 20 times, 740. The value for a 4.0 per cent solution of pure α-naphthalene sulfonic acid (0.5N) was 950, and for pure β-naphthalene sulfonic acid (0.5N) was 2030. It was experimentally proved that this increased penetrability was not due to a solvent action of the syntans upon the hide substance. When the hide through which the syntan solutions had passed was washed with water until the great bulk of the syntan present in the hide had been removed, its permeability fell far below 100, the value for the original pure water control. The pH values of the various syntan solutions described above were all less than 2.0. Acid solutions, such as hydrochloric, of such pH value would have tremendously swollen the hide and thus decreased its permeability. Experiment showed, however, that all the syntan solutions swelled the hide less than did pure water.

The permeability experiments described above should be extended and should include permeability values for various tannin solutions, alone and in admixture, together with the effect of added syntans.

Compton[27] recently studied the effect of the addition of a naphthalene sulfonic acid syntan to quebracho solutions upon the retained water of steer hide tanned in such admixtures. By "retained water" is meant water which cannot be removed from the leather under pressure in a hydraulic press, but which can later be removed by vacuum-drying. He found that the presence of this syntan in the quebracho solution caused the retained water of the leather to be held much more firmly than in its absence.

Effect of the Liming of Hide Substance on Tannin Fixation

Since the character and the degree of liming treatment may have a marked effect on the subsequent tanning behavior of a hide or skin, Gustavson and Widen[44] endeavored to determine the effect of liming time on tannin fixation. They soaked pieces cut from the butt of salted steer hide in water for 12 hours and then limed them for varying time periods in a mixture of calcium hydrate and sodium sulfide. After unhairing, washing, and deliming, the hide was brought to a pH value of 5.0. The unlimed control was obtained by removing the hair and epidermis from the soaked hide by means of a razor; thus the control was really corium, and not whole hide. The limed specimens, together with the soaked but unlimed corium control, were then cut into pieces 4.0 mm long. Six grams of the various specimens were then tanned for 48 hours, with constant agitation, in 100 ml of hemlock bark extract having a pH value of 4.18 and containing 6.7 grams of total solids per liter. After this tanning period 100 ml of the same tanning solution, but containing 20.1 grams of total solids per liter, were added, and the tanning was continued for five days. The specimens were then washed and analyzed for combined tannin. The unlimed corium control contained 26.5 parts per 100.0 parts hide substance, the hide limed for 12 hours, 27.7 parts; that limed 7 days, 33.6 parts; and that limed 14 days, 35.7 parts. Even though the corium control specimen was not strictly comparable in liming and subsequent tanning behavior with the hide pieces, the results on the full hide specimens demonstrated that the degree of liming was of importance in subsequent tanning. Practical tanning experience demonstrates the important bearing which liming has on tanning. Gustavson and Widen commented on the possible explanation of their results as being related to both the chemical change brought about by liming—manifested by activation of both acidic and basic protein groups—and also to the effect of liming in increasing the active surface of the hide.

Page and Holland[76] soaked butt pieces of salted cow hide, then mechanically removed flesh and epidermis, and used the resulting corium throughout for the following experiments:

The specimens were limed in pure calcium hydroxide solutions containing excess lime for the time periods shown; after this they were delimed and then

Table 255

Duration of Liming	Combined Tannin (%) (a)	Combined Water-solubles (%) (b)	(a) + (b)
Not limed	34.6	31.4	66.0
Limed 14 days	33.1	44.9	78.0
Limed 1 month	30.0	44.9	74.9
Limed 2 months	29.4	48.0	77.4
Limed 3 months	36.3	51.2	87.5

tanned for six weeks in increasing concentrations of wattle bark tannin at a pH value of 5.0. The resulting leather was air-dried and analyzed. The results are shown in Table 255, wherein fixations on hide substance basis are given.

From the experiments of Page and Holland on corium we note that the amount of irreversibly fixed tannin is not greatly affected by liming, or the length of liming time, but that liming effects on the combined water-solubles may be very great.

Wilson and Daub[132] placed the soaked butt of a calfskin in saturated lime solution and histologically followed its structural changes, as a function of time, up to seven months' contact. Very drastic structural changes were found after four weeks; and it is a matter of tanning experience that the longer liming is continued after the objects of the liming treatment have been attained, the poorer the quality of the leather will be, and the lower its yield.

Effect of Pretreatment of Hide Substance with Neutral Salts on Tannin Fixation

Hides and skins are subjected to the influence of neutral salts in curing and soaking and to some extent in liming. Gustavson[43] therefore investigated the tannin-fixation power of hide powder which was subjected to treatment with a wide variety of neutral salts prior to vegetable tanning. He soaked 100 gram specimens of hide powder in a liter of molar concentration of a great variety of neutral salts for 14 days, at pH values between 5.7 and 6.0. Toluene was added to prevent bacterial growth. Nitrogen was determined in the residual salt solution, and it was found that the salts varied greatly in their ability to dissolve the collagen; sodium sulfate, for example, dissolved only 1.8 per cent of the original collagen, whereas calcium chloride dissolved 31.9 per cent and the distilled water control dissolved 4.9 per cent. The salt-treated powders were then washed salt-free, dehydrated with alcohol, and air-dried. They were then tanned for 48 hours at pH 4.1 with hemlock-bark extract, after which all soluble matter was washed out and the fixed or combined tannin determined. This value also varied greatly: the sodium sulfate specimen fixing 40.1 parts tannin per 100.0 of hide substance, the calcium chloride specimen, 78.9, and the water control, 56.8.

Since these experiments of Gustavson's were made with hide powder, Merrill[63] repeated them, employing strips of bated calf skin, because this material more nearly represents actual tannery conditions. He soaked the bated skin for 20 days in normal solutions of sodium chloride, sodium sulfate, and calcium chloride, toluene being added to all three solutions. The strips were then washed free of salt and were tanned for six days in an oak-bark extract solution containing 20 grams of solids per liter. They were then rinsed, dried, and analyzed for fixed tannin and showed: water control, 39.6 parts

fixed tannin per 100 parts of hide substance, sodium chloride treatment, 39.1 sodium sulfate, 39.9, and calcium chloride, 48.6.

Page and Holland[76] investigated the effect of calcium chloride upon the *corium* of salted cow hide in relation to both combined tannin and combined water-solubles when subsequently tanned with wattle bark at a pH value of 5.0. Their results are shown in Table 256. The values shown are the percentages based upon hide substance.

Table 256

Treatment	Combined Tannin (%) (a)	Combined Water-solubles (%) (b)	(a) + (b)
No Pretreatment	34.6	31.4	66.0
Pretreated 14 days in M $Ca(Cl)_2$	29.0	44.2	73.2
Pretreated 2 months in M $Ca(Cl)_2$	29.6	44.0	73.6

We note the very considerable effect of calcium chloride on the tanning behavior of hide substance. It is as yet impossible to state whether this is the result of a chemical change in the hide substance or of a change in its reactive surface.

Effect of Pretreatment of Hide Substance with Acid or Alkali on Tannin Fixation

Gustavson[46] treated hide powder with acid and with alkali so as to subject it to the action of a pH range of 1.0 to 14.0; after 24 hours of such treatment, he brought all specimens to pH 5.0. They were then thoroughly washed, dehydrated with alcohol, and dried. The treated, dried powders were then tanned for 48 hours with hemlock extract at a pH value of 4.0. The specimens were thoroughly washed after tanning, and the fixed tannin was determined. Great differences in tannin fixation were found; when these values were plotted against the pH values of the pretreatment, a curve was obtained which was almost identical with that secured when the swelling of collagen is plotted against the pH values of the swelling solutions.

Thomas and Kelly[113] repeated Gustavson's experiments, except that they employed oak bark extract at pH 4.1 as the tanning agent. When the pretreated powders were introduced into the tanning solution in a dry state, they confirmed Gustavson's results, in general. But when the pretreated powders were first wet with water before tanning, there was no difference in tannin fixation throughout the entire pH range. Merrill[63] soaked strips of bated calf skin in $0.1M$ hydrochloric acid, in $0.1M$ sodium hydroxide, and in distilled water for 24 hours at 7°. The strips soaked in the acid and in the alkali were of course greatly swollen. All three specimens were then brought to a pH value of 4.5 and tanned in regular tannery liquors, after which they were dried, and the combined tannin determined. The acid-treated strip contained

44 parts combined tannin per 100 parts hide substance; the alkali-treated, 43; and the distilled water control, 44. In other words, the pretreatment had no effect on the ability of the skin to fix tannin.

In considering the above experiments and their negative results, it must be remembered that both hide powder and bated skin have already been subjected to a pH value of approximately 12.5 during liming. Such treatment may well obviate any of the pH effects which Gustavson sought. Fortunately, Page and Holland[76] conducted similar experiments, employing the corium of soaked salted cow hide from which the flesh and epidermis were removed by mechanical means. In this way experimental material was obtained which had not been subjected to the effect of liming. Corium specimens were treated with 0.1*N* hydrochloric acid and with 0.1*N* sodium hydroxide solutions as shown, were then neutralized and washed free of soluble salts, and were tanned for six weeks in increasing concentrations of wattle-bark extract at a pH value of 5.0. The leather was then air-dried and analyzed, with the results shown in Table 257, wherein the values are based on hide substance.

Table 257

Treatment	Combined Tannin (%) (a)	Combined Water-solubles (%) (b)	(a) + (b)
No pretreatment	34.6	31.4	66.0
Soaked 14 days in alkali	32.6	42.8	75 4
Soaked 2 months in alkali	34.0	42.5	76.5
Soaked 14 days in acid	28.7	47.5	76.2
Soaked 2 months in acid	31.0	46.2	77.2

The figures in Table 257 indicate that either acidic or alkaline pretreatment of unlimed corium slightly decreases its capacity for fixing tannin, under the experimental conditions described, but that both pretreatments increase the combined water-solubles. When the combined tannin and combined water-solubles are added together, they show a constant value for all the pretreatments, and this value is considerably greater than that for untreated corium.

Effect of Deaminization

When collagen is deprived of a portion of its basic groups by deaminization, it should behave differently toward vegetable tannins; that is, it should fix less tannin if the mechanism of tanning is a combination of the tannins with the protein basic groups. The deaminized collagen should also show a different and more acid isoelectric point. These logical assumptions led Thomas and Foster[114] to investigate the behavior of hide substance which they deaminized according to the procedure of Hitchcock.[50] The deaminized hide powder used in their experiments contained 17.32 per cent nitrogen; the original powder showed 17.81 per cent and the difference, or 0.49 per cent,

was taken as the index of the extent of deaminization. Two-gram portions of the two powders were tanned for 24 hours at room temperature and with constant agitation with 200 ml of the materials shown in their table, which is here reproduced as Table 258.

Table 258. Fixation of Tannins by 2 G. of Deaminized Hide Substance. Time of action, 24 hours.

pH of tanning soln.	Wattle-bark Ex T.s.[a] = 41 1 g./l D.h.p.[b]			Quebracho Ex T.s. = 26 4 g./l D.h.p.		Hemlock-bark Ex T.s. = 58.2 g./l D.h.p.	Gambier Ex T.s. = 32 5 g./l. D.h.p.	Oak-bark Ex T.s. = 34 4 g./l D.h.p.
	D.h.p.[b]	[d]	O.h.p.[c]	D.h.p.	O.h.p.			
1.0	0.86	1.48	1.55	0.89	1.16	0.40	0.26	0.35
2.0	.67	1.10	1.46	.52	1.29	.50	.17	.47
3.0	.44	0.90	1.12	.50	1.12	.32	.16	.32
3.5	.41	.87	0.99	.53	0.87	.33	.19	.29
4.0	.47	.94	.65	.55	.69	.38	.19	.33
4.5	.54	1.03	.62	.57	.67	.47	.21	.38
5.0	.58	1.14	.52	.65	.50	.54	.24	.41
6.0	.69	1.22	.56	.61	.57	.59	.26	.42
7.0	.82	1.40	.59	.75	.58	.62	.30	.44
8.0	.88	1.59	.76	.74	.75	.62	.29	49
9.0	.56	0.64	.70	.38	.49	.41	.26	.27
10 0	.19	.28	.37	.10	.20	.17	.04	.10

[a] T.s. = total solids.
[b] D.h.p. = deaminized hide powder.
[c] O.h.p = ordinary hide powder.
[d] Values in this column are for two weeks' action.

The table indicates a decreased tannin fixation by the deaminized powder at pH values less than 4.0 compared with regular powder, but occasional greater fixation from pH 4.0 to 8.0. The pH value of the isoelectric point of regular powder is around 5.0, and that of the deaminized, as determined by the dye technique, between 3.7 and 4.2. Thomas and Foster explained that the lessened fixation in the range pH 1.0 to 4.0 was governed by the shift shown in the isoelectric point. On the other hand, they found that the increased fixation from pH 4.0 to 8.0 was correlated with an increased plumping capacity of deaminized calf skin in this pH range. The suggestion was also made that the phenomenon may be related to an increased amount of beta collagen which deaminization may have brought about.

The term "beta" refers to the second of two forms of gelatin or collagen postulated by Wilson and Kern,[132] who found calf skin to have two points of minimum plumping capacity: one point at pH 5.1; and the other at 7.6.

The experiments referred to above were made with hide powder, and the fixed tannin was obtained by means of the Wilson and Kern method whereby, as we have noted, only the difficultly reversible fixed tannin was determined. Page and Holland[76] deaminized pieces of cowhide which had passed through beamhouse processes and were tanned, together with undeaminized controls, for six weeks with wattle-bark tannin at pH 5.0. At completion of tannage,

the leathers were analyzed for both firmly fixed substances, designated as combined tannin, and easily reversible fixed substances, designated as "combined water-solubles." Their results are shown below, where the combined tannin and combined water-solubles are expressed as percentage combined with hide substance.

	Deaminized H.S	Control H S
Per cent combined tannin	22.5	33.1
Per cent combined water-solubles	40.7	42.1
	63.2	75 2

Page and Holland commented that these figures indicate that the lowering of combined tannin was brought about by deaminization, as Thomas and Foster had shown, but that the combination capacity of combined water-solubles was but slightly changed, which they interpreted to mean that basic protein groups were not involved. In a later study, Page and Holland[78] tanned both deaminized and regular hide powder with wattle-bark tannin and determined the amount of fixed tannin resistant to hydrolysis and removal when the tanned powder was washed with dilute alkali solution. They found the deaminized tanned powder to contain considerably less alkali-resistant tannin. They believed that it was uncharged NH_2 groups which were removed by deaminization.

Lollar[59] has recently studied the comparative take-up of quebracho tannin by regular and deaminized hide powder, employing both the A.L.C.A. analysis method and the Löwenthal method for determining residual tannin. Deaminization was found to decrease the tannin take-up at all pH levels to the value found at pH 4.8 for ordinary hide powder.

Stiasny[106] has pointed out and stressed that the peculiar behavior of deaminized hide substance compared with undeaminized may, with equal certainty, be attributed to a change in its structure, whereby its active surface has been decreased, rather than to purely chemical modifications.

Enzymatic Digestion of Vegetable-tanned Leather

One of the most commonly used descriptive criteria for leather is its resistance to enzymatic digestion, in contrast to untanned hide substance. Strangely enough, however, the literature contains very little information on this very important point.

Thomas and Seymour-Jones[112] studied the action of trypsin on hide powder, and their results may be stated as follows:

Untanned hide powder was rapidly digested by trypsin, the optimum pH value for the hydrolysis being approximately 5.9. The rate of hydrolysis was a function, among other factors, of the size of the substrate particles, increasing as the size of the hide-powder particles diminished, indicating that the action

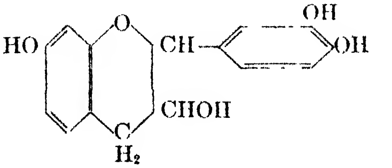
was taking place at the surfaces of the particles. Finely sifted hide powder (passing a 100-mesh sieve) was tanned with basic chrome sulfate, quinone, formaldehyde, and gallotannin respectively. The leathers were thoroughly washed to remove uncombined matter and were then dried, and the dry leather was then digested with trypsin. The results may be summarized: when collagen was completely chrome-tanned, it was not hydrolyzed by trypsin; quinone-tanned collagen was readily hydrolyzed, as was formaldehyde-tanned. The dry hide powder tanned with gallotannin contained some 30 per cent combined tannin on the hide substance, and this showed a very considerable hydrolysis by trypsin.

Bergmann[7] extensively studied the action of trypsin on collagen and found that in the case of fresh collagen fibers, only the *exterior surfaces* were attacked by the enzyme. He pointed out that when collagen fibers were limed and then delimed, trypsin could then penetrate them and digest the inner as well as the outer surfaces. (This finding has an important bearing on bating, as we have seen.) Bergmann studied the digestion of various leathers with trypsin, and the results are shown in Table 259.

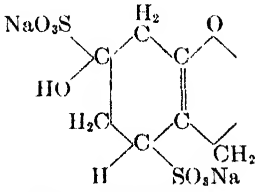
Table 259

Kind of Leather	H S. digested with trypsin (%)
Belting leather, pit-tanned	15.4
Belting leather, drum-tanned	26.2
Leather tanned with ordinary quebracho	33 6
Leather tanned with sulfited quebracho	41.6
Leather tanned with ordinary quebracho plus —naphthalene sulfonic acid	54.6
Chrome-tanned leather	3.1

Bergmann ascribed the difference in trypsin indices between leather tanned with ordinary quebracho and with the sulfited quebracho to the change in composition of the quebracho tannin which was induced by sulfiting, as the formulas below show.



Quebracho-Catechin



Effect of Sulfiting

It is to be hoped that this subject will be more fully investigated, with leather rather than tanned hide powder, since such data should prove helpful in extending our understanding of the vegetable-tanning mechanism. The

data should include complete analysis of the leather before and after treatment with enzymes.

Effect of Vegetable Tannage on the Acid-combining Capacity of Hide Substance

This subject has a two-fold interest. From a theoretical standpoint, it is important to know whether both tannin and acids combine with the same skin protein groups. From a practical standpoint, the question bears upon the destructive action of acids upon leather, particularly sulfuric acid. We are here more particularly concerned with the theoretical phase of the problem.

Wilson and Bear[131] tanned hide powder with oak-bark extract so as to secure a wide range of combined tannin. They then washed the tanned powders free of all soluble tannin matter, and any combined acid they may have contained, and dried them. An amount of each tanned powder equivalent to 1.0 gram of hide substance was covered with 50 ml 0.01N sulfuric acid. This mixture was shaken at intervals for 24 hours at room temperature, when equilibrium was reached. The pH values of the acid solutions were then electrometrically determined, with the results shown in Table 260.

Table 260

% Combined Tannin per 100 gms H S	pH Value of Acid Solution	% Combined Tannin per 100 gms H S	pH Value of Acid Solution
0.00	3.39	12.17	2.96
6.84	3 37	12.61	2.86
7.59	3.23	14.24	2.80
7.86	3.14	17.47	2.72
10.45	3.07	Original acid solution	2.05

These results indicated that tannin and sulfuric acid did combine with the same protein basic groups, since the amount of unabsorbed acid left in the solution, as indicated by the pH values, increased as a function of the tannin combined with the protein. In view of this finding, Gustavson reasoned that if tannin did combine with the basic groups of hide substance, its isoelectric point should be shifted to the acid side. He tanned two specimens of hide powder: one with hemlock-bark extract to contain 49.8 parts combined tannin per 100.0 parts hide substance after washing to remove all soluble matter, and the other to contain 30.9 parts combined tannin from quebracho extract. The moist, undried leathers were then treated for 12 hours with buffer solutions of the desired pH values, at pH intervals of 0.2. This was followed by a six-hour treatment in fresh buffer solutions, to which 2 ml of 1.0 per cent dye solutions were added. The dyestuffs used were acid blue, acid magenta, and Orange II. The highest pH value at which any dye fixation occurred was taken as the isoelectric point of the leather. This

value was found to be 3.9 for the hemlock leather and 4.0 for the quebracho. The isoelectric point of the untanned hide powder was 5.0.

Mieckeley,[68] however, obtained results exactly opposite to those of Wilson and Bear. He tanned hide powder for 14 days with filtered infusions of ordinary quebracho, sulfited quebracho, chestnut wood, oak bark, pine bark, oak/pine barks mixed, and sulfite cellulose—all at their natural pH values. The tanned powders were then washed free of all soluble matter and dried. They were then analyzed for combined tannin which was found to vary from 31.7 parts per 100.0 of hide substance in the case of the pine bark to 36.2 parts for the chestnut wood. Two grams of the dried tanned powder were then treated for varying time periods with 50 ml of $0.1N$ sulfuric acid. At the end of the acid treatment the mixture was centrifuged, and the acid remaining in the solution was analytically determined. The results indicated that, with the exception of the sulfite cellulose leather, the combined tannin of the powders had not interfered with their acid-combining power; this was practically the same as in the case of untanned hide powder. From these experiments, Mieckeley assumed that tannin did not combine with free basic protein groups; he believed it to be predominantly fixed at the peptide linkages.

Thompson[121] made the very interesting statement that tannin will quantitatively displace hydrochloric acid which is combined with gelatin or hide substance. As proof of this contention he performed the following experiments.

Five-ml portions of 0.4 per cent ash-free gelatin chloride were brought to the pH values shown in Table 261. These various gelatin specimens were

Table 261

pH value of original gelatin	pH value of original tannin	Final pH value found	Final pH value calculated
4.17	4.17	4.02	4.02
3.28	3.92	3.58	3.60
3.48	3.92	3.63	3.65
3.55	3.92	3.66	3.67

then added to 100 ml of 1.0 per cent purified tannin solution brought to the pH values shown. After equilibrium was reached, the pH values of the mixtures were determined and were found to equal exactly the theoretical pH of the mixtures, assuming that *all* the acid combined with the gelatin had been displaced by tannin and had been forced out into the surrounding tannin solution. Thompson considered this to be strong evidence that tannin and acid combine with the same protein groups.

This would seem to be a very important finding, and we have tried to confirm it but have been unable to do so. We brought 5 ml of 0.4 per cent

ash-free gelatin which showed a pH value of 4.70 to the following pH values by the addition of 0.1N hydrochloric acid: 4.20, 3.90, 3.60, 3.30 and 3.00. This required the addition of 0.1N acid as follows: 0.02, 0.045, 0.065, 0.087, and 0.115 ml respectively. Each of these 5.0-ml gelatin solutions was added to 100 ml of tannic acid at a pH value of 4.20, and each mixture was vigorously shaken. At the end of two hours the pH values were determined with the glass electrode and were found to be: 4.20, 4.20, 4.20, 4.20, and 4.20; these values were unchanged at the end of 21 hours. In other words, no detectable amount of hydrochloric acid was displaced from the gelatin by the tannic acid. In order to change the pH value of 100 ml of tannic acid from, say, 4.20 to 4.01 required, we found, 1.0 ml of 0.1N hydrochloric acid; 5.0 ml of 0.4 per cent gelatin solution required the addition of 0.025 ml of acid to bring it from 4.7 to 4.2. The addition of the 0.025 ml of acid from the gelatin (assuming it was all displaced) to the surrounding tannin solution would cause a practically undetectable change in its pH value.

In view of the very conflicting results obtained by the several workers noted, this subject should be further investigated, and we suggest the following procedure. Unhaired and delimed hide should be treated with acid and all the excess, uncombined acid pressed out in a hydraulic press at an actual pressure of not less than 5000 lbs per square inch, and the acid actually combined with the hide substance determined. Pressed specimens containing only combined acid should then be tanned in various tannin solutions, and the amount of acid leaving the skin may be quantitatively determined in the surrounding tan liquor. Duplicate experiments should be run in which the pressed, acid-treated hide is subjected to the action of a similar volume of distilled water. The difference in value of the acid found in the tan liquor and in the water control would indicate to what extent the phenomenon is one of simple hydrolysis and to what extent tannin replaces combined acid.

The experiments of Innes,[54] which indicated the displacement by tannin of combined sulfuric acid from hide, were not conclusive, since his hide specimens were partially tanned before they were subjected to acid treatment.

X-ray Examination of Vegetable Leather

Modern investigations of protein structure have been advanced by means of x-rays. In view of this, numerous x-ray studies of leather have been undertaken, in the hope of throwing light on the nature of tanning. While as yet these studies have added little or nothing of a fundamental nature to our knowledge, this does not mean that they may not in the future, as both techniques and interpretations are improved.

In 1926, Katz and Gerngross[56] examined various leathers, employing disks 1 mm thick cut parallel to the grain surface. These yielded a diagram having a broad amorphous inner ring and a very narrow outer ring, the

diagrams of the various leathers being the same except as the intensity of the rings varied. Using pine bark, quebracho, oak bark, and formaldehyde as tanning materials, they next examined sinews in the raw state and again after they were tanned and dried. The diagrams obtained showed no change from the untanned sinew, except in the case of the oak bark. They expressed surprise that the formaldehyde-tanned leather showed no change, since formaldehyde was presumed to react chemically with the collagen basic groups, and they suggested that the formaldehyde may have reacted with micelle surfaces only, leaving the interior untanned and therefore unchanged.

Chesly, Anderson, and Theis[23] made x-ray examinations of animal skin which had been subjected to beamhouse processes but were unable to find any structural change during curing, soaking, liming, or bating. Thuau[122] was also unable to find changes in the diagram of dry skin before and after soaking, or in skin tanned with formaldehyde, mineral, or vegetable agents. In the case of these leathers the diagrams were essentially the same as before tanning, except for the addition of the superimposed diagram of the tanning material.

Highberger and Kersten[47] have examined a great variety of leathers. They have found that no change in the collagen x-ray diagram was brought about by tannage with formaldehyde or with the syntan they employed. They did find a definite definition loss in the case of quinone, vegetable, and chrome tanning; they ascribed this to an actual distortion of the collagen fiber structure due to the introduction of the tanning agent. They pointed out that when their samples of both vegetable and chrome leathers were detannized, the diagrams obtained were identical with that of the original untanned collagen.

Lloyd[68] compared the diagrams of collagen before and after vegetable tanning, calling attention to the definition loss of the ring due to the side-chain spacing, which she felt proved that vegetable tanning took place at the active centers of the side chains. She further pointed out that in good-quality leathers the ring due to side chain spacings became diffuse, but that the rings due to the backbone spacings and the backbone units were as clear as in collagen. This was not the case, however, with poor-quality vegetable leathers, where all of the rings lost definition. Pursuing Lloyd's suggestion, Bowker and McNicholas[14] made x-ray diagrams of numerous samples of leather tanned with quebracho and with chestnut—both of good quality and of quality so poor that the leathers could be pulverized by hand, due to sulfuric acid added in tanning. The diagrams showed no difference in the two kinds of leather, except that the poor quality showed an increased definition of the outer ring—in other words, just the opposite of what Lloyd had found. They noted that greater differences in x-ray diagrams were found as

a function of different materials used in tanning rather than of good or poor leather tanned with the same material.

Astbury[3] has pointed out that collagen fibers differ greatly among themselves and that no two x-ray diagrams of collagen fibers are strictly alike. Highberger and Kersten[48] in a later study have found that most of the changes brought about in collagen x-ray diagrams by tanning agents may be induced by mere mechanical treatment, such as prolonged grinding or pressure.

Reversibility of Tannin Fixation

A great deal of work and argument has centered around the question of just how reversible is the reaction between hide substance and tannin. This problem is of great importance, since it not only bears in a significant manner upon the practical production and wearing qualities of leather, but constitutes an important phase of tanning theory as well.

The practical tanner has long known that in tanning hides or skins they must always be treated with increasingly stronger tan liquors, since otherwise they will "go back." This was illustrated by Earp[32] in 1907; he stated: "I put a butt which had been a long time in one valonia liquor into a weaker liquor, and I was much struck with the loose and unfilled appearance which the butt had after a few days in the weaker liquor. I tried my best to make the butt recover its plumpness, but could not do so." On the other hand, and to illustrate the probability that the collagen/tannin compound is never completely hydrolyzed, we may recall the report of Churchill[24] who, in 1919, analyzed sole leather recovered from a ship sunk in Lake Huron in 1865. After some half-century of contact with cold water, the leather showed 13.11 per cent of combined tannin on hide substance.

As one digests the voluminous literature of reversibility of the past twenty years, it becomes apparent that the numerous, and often conflicting, data do not permit final conclusions. At the same time much valuable knowledge has been gained.

The Wilson and Kern method of tannin analysis resulted from these workers' dissatisfaction with the official method because it did not differentiate between that tannin which they felt to be irreversibly fixed by hide substance and that loosely fixed or not really fixed at all; and they believed that the irreversibly fixed tannin which a material imparts to hide substance was the true, and only real measure of its tanning value. All the modern studies of reversibility were stimulated by the arguments started by the Wilson and Kern analysis method. This method consisted, as has been already stated, in agitating for six hours at room temperature 2 grams of hide powder with 100 ml of filtered analytical concentration solution of the tanning material which was examined. The mixture of powder and liquor was then washed into a special glass extractor designed for the purpose, and distilled water was

passed through the tanned powder until the wash water gave no tannin test with a solution of 10 grams of gelatin and 100 grams of sodium chloride dissolved in distilled water and made up to a liter. At this point all the reversible tannin was presumed to have been removed, leaving behind the irreversibly combined. The washed powder was carefully dried and weighed; the difference in weight found between the powder before and after tanning, washing, and drying represented the percentage of tannin in the material examined. Table 262 shows the difference in percentages of tannin found by the Wilson and Kern and by the official method.

Table 262 Percentage Analysis of Material.

		A L C. A Method			Wilson-Kern Method
		Insoluble	Soluble Matter		
Material	Water	Matter	Nontannin	Tannin	Tannin
Quebracho	17.87	7.16	6.96	68.01	47.41
Hemlock bark	8 90	74.33	6.71	10.06	6.17
Oak bark	52.66	3.68	19.46	24.20	12.88
Larch bark	51.08	5.88	20 90	22.11	11.71
Chestnut wood	58.90	1.50	13.80	25.80	11.90
Sumac	9.25	47.20	17.99	25 56	9.61
Osage orange	46.05	3.45	10.63	39.87	13.37
Gambier	51.12	5.36	18 57	24.95	7.79

The Wilson and Kern method has proved of value as a research tool and as a stimulus to further work, but it has not replaced the official method, which more nearly determines the actual value of a tanning material and the behavior of tannery liquors, as shown by Schultz and numerous other workers.

Page[74] and Page and Holland,[75] in a series of papers starting in 1928, attempted to differentiate between "free water-solubles," "combined water-solubles," and "combined tannin." They made many experiments of washing for long periods leather tanned with wattle-bark tannin and analyzed the wash waters for dissolved tannin and non-tannin. They found that regardless of the length of the washing period, the water continues to remove soluble matter. But when the dissolved values were plotted, the curve showed a somewhat sharp break, at which point the rate of removal of soluble matter dropped to a very low value, the exact amount of the drop varying with the pH value of the water used for extraction. The material removed before the break in the curve comprised both free and combined water-solubles. The combined material which remained in the leather after all the water solubles had been removed was the combined tannin. To differentiate the free and the combined water-solubles, they suggested shaking 10 grams of the leather with 100 ml of water for eight hours and determining the matter dissolved, which equaled the free water-solubles. This value deducted from that obtained for the total water-solubles (*i.e.*, the value obtained before the break in the curve) equaled the combined water-solubles. They suggested that the combined water-solubles represented tannin combined with the hide

substance in some manner different from that in which the combined tannin was fixed.

Merrill[64] also studied the hydrolysis of leather with water and stated that such studies enabled him to distinguish three classes of materials present in leather: (1) substances not combined with collagen, (2) substances loosely combined, and (3) substances combined to form compounds that were practically non-hydrolyzable. (This classification is essentially the same as that of Page.) Merrill added that the line of demarkation between the three classes was not sharp. He performed washing experiments with hide powder and with hide cubes tanned with various tanning materials. As a result of these experiments he found that such materials as wattle, quebracho, and oak bark formed compounds with hide substance which were extremely resistant to hydrolysis, together with compounds which were more or less easily removed by washing with water, but that gambier tannin appeared incapable of forming a hydrolysis-resistant compound with hide substance. Accurate knowledge of the particle size of these contrasting materials when dissolved in water would be very important. The general subject of reversibility of tannin fixation by water has also been discussed by Stather and Lauffmann,[100] Colin-Russ,[26] Forman and Thompson,[35] Pound and Quinn,[85] A. Cheshire,[22] and by Braybrooks, McCandlish and Atkin.[15]

While recognizing that the methods discussed above are empirical, it is to be realized that the difficulties involved are so great that it is doubtful if any really clear-cut differentiation of the various stages and kinds of fixation will be achieved. It is of interest to note, however, that Marriott[62] studied the refractive indices of leather fibers and concluded that tannin seems to be held in three distinct ways: (1) fixed or combined tannin which is not removed by washing but which affects the refractive index to a small extent; (2) a fraction of water-soluble material not chemically combined but intimately associated with the tanned fiber and contributing to the refractive index; (3) a further portion of water-soluble material present as a coarse admixture, which does not affect the refractive index. The latter material probably coats the fibers and fibrils or is scattered throughout the structure of the tanned leather, but does not penetrate into the micellar or ultramicroscopic structure of the fibers.

The reversals reviewed above are those in which distilled water was employed. We shall now discuss other solvents.

Wilson and Kern[132] investigated the reversal of combined tannin by means of acidic and alkaline aqueous solutions. Hide powder was tanned with ordinary quebracho extract at pH 4.6, washed with distilled water until all easily soluble matter was removed, and then dried and analyzed; and it showed 18.53 per cent "combined tannin" on hide substance basis. Buffer solutions were prepared from $0.1N$ phosphoric acid with sodium hydroxide

to give pH values of 5, 6, 7, 8, 9, 10, and 11, respectively. Eight-gram portions of the dry leather were then extracted for six hours with four liters of each of the buffer solutions; that is, each portion was extracted with a solution of different pH value. The extracted leathers were then washed free of buffer solution with distilled water and were dried and analyzed. The results showed that buffers of pH 5, 6, and 7 had extracted none of the combined tannin, but that buffers of pH 8, 9, 10, and 11 had extracted 2.6, 3.8, 6.4, and 8.3 per cent of the original combined tannin, respectively.

Marriott[61] has extended the studies of Wilson and Kern. Commercial leathers and also leathers made from hide powder tanned with various materials were washed with distilled water to remove all soluble matter and were then dried. The dried leather was then extracted with alkaline solutions, *i.e.*, solutions of $0.1N$ and $0.05N$ sodium carbonate and also saturated borax solution. The dried (water-soluble free) leathers were extracted with the various alkalies up to 96 hours at both 15/20° and at 42°. The general result indicated that from 9 to 12 per cent of tannin remained combined with the hide substance after such alkaline extractions. Marriott gives an interesting discussion of his experiments but feels that any adequate explanation of the manner of combination between alkali-resistant combined tannin and collagen cannot be given at present. It is significant that Marriott found that the amount of combined tannin resistant to alkaline treatment is essentially the same, whether the leather is or is not dried out before extraction.

Page and Holland[78] have employed Marriott's method in the examination of hide powder tanned for seven days with wattle-bark extract, the pH values of which ranged from 2.0 to 11.0. The hide powder specimens were soaked for 24 hours before tannage in aqueous solutions having the same pH value as that of the tan liquor which they entered. After tanning, the powders were drained and were air-dried at room temperature. They were then analyzed according to Page's methods described on page 605, and to these results was added the value for irreversibly combined alkali-resistant tannin according

Table 263

pH Value of Tannage	2.0	5.0	5.0*	8.0	8.2	8.4
% Comb. Tannin on H.S.	84.0	42.9	34.1*	76.4	148.3	160.9
% Comb. Water Sol. on H.S.	108.1	66.4	67.9*	83.6	19.1	8.2
% Irreversibly Comb. Tannin on H.S. Marriott's Method	30.2	18.1	6.8*	23.6	50.1	80.9

pH Value of Tannage	8.5	8.5**	8.6	8.8	9.0	9.5	10.0	11.0
% Comb. Tannin on H.S.	149.7	72.9**	130.0	127.5	57.8	60.5	51.7	30.9
% Comb. Water Sol. on H.S.	22.7	28.5**	11.5	8.3	24.1	4.6	5.7	2.6
% Irreversibly Comb. Tannin on H.S. Marriott's Method	60.5	22.0**	60.7	93.6	27.8	31.3	37.9	30.1

* Deaminized Hide Powder.
** Air Excluded.

to Marriott's method, using $0.1N$ sodium carbonate and treating for 24 hours at 42°. The results are shown in Table 263. All the figures of the table refer to tannage in the presence of air except those at pH 8.5, at which value they found the presence or absence of air had great effect; this striking difference was not found at any of the other pH values.

As has long been known, tannins dissolve to varying degrees in certain organic solvents. Such solvents have been employed in the study of the reversibility of combined tannin in leather.

In 1910, Trunkel[123] showed that the water-insoluble compound of gelatin and tannin could be resolved into its original components when digested with alcohol, if the treatment was given before the precipitate was dried; after drying, the precipitate was unaffected by ethyl alcohol. With these phenomena in mind, Thomas and Kelly[117] investigated the behavior of tanned hide powder toward alcohol. One-gram portions of hide powder were tanned with solutions of hemlock-bark extract and of gambier, all of which had been brought to pH values of 1, 3, 5, 7, and 9, respectively. The tanned powders were then washed with distilled water until free of soluble matter. The tanned *wet* powder was then placed in a Thorn extractor and extracted with 95 per cent ethyl alcohol. The alcohol was evaporated, and the residue weighed; the amount of alcohol dissolved tannin was calculated as the percentage of the original combined tannin which the alcohol removed. The results for the hemlock tannage are shown in Table 264.

Table 264

Tanned at pH Value of	Grams Tannin Fixed by 100 Grams H S	Per cent of Total Fixed Tannin Removed by— Extraction for 1 Hour	45 Hours	91 Hours
1.0	59.1	6.1	19.3	23.3
3.0	47.4	7.7	24.1	28.9
5.0	16.6	7.8	17.1	22.0
7.0	33.3	3.1	6.9	9.8
9.0	28.4	4.2	6.3	8 4

When the above experiment was repeated—but with tanned powder *dried* before alcohol extraction—the amounts of combined tannin removed were negligible; this confirmed Trunkel's observation. In the case of the gambier-tanned powders, the alcohol-extracted matters were obtained by weighing the leather (and not the residue from the evaporated alcoholic solution) after drying. These results were less reliable, since the last specimen of the series (the one tanned at pH 9.0) showed a *gain* of 13.8 per cent in weight. Thomas and Kelly suggested that this may have resulted from the oxidation of the alcohol to aldehyde which may, in turn, have combined with the collagen.

Page[77] has studied the comparative stripping action which various solvents have on hide powder tanned with a specially purified wattle-bark tannin

which was completely soluble in all the solvents employed for stripping. The tanned powder was pressed and air-dried; it was not washed. Ten-gram portions of this tanned hide powder were extracted for 24 hours with successive 100-ml lots of the various solvents. If we designate the total amount of material extracted by distilled water as 100 and relate thereto the total removed by the other solvents, the following values are found:

Methyl alcohol, 141; ethyl alcohol, 125; acetone, 109; acetic acid, 82; isopropyl alcohol, 12; and ethyl acetate, 10. Page noted that the order of stripping effectiveness of these solvents was, with the exception of water and isopropyl alcohol, the order of magnitude of their dielectric constants.

Stather, Lauffmann and Bau Miau[101] found that the solubility of various tanning materials in a given solvent varied greatly, as did the solubility of a given material in different solvents. The results suggested, however, that only those solvents with alcoholic OH groups have marked solvent powers for most of the materials. These authors believed decreasing solvent action may be related to decreasing polarity of the solvents. The polarity of the tanning material itself influences solubility capacity; for example, acetone dissolves 82 per cent of ordinary quebracho but only 26 of sulfited. These workers found an aqueous mixture of 25 or 50 per cent of a solvent such as methyl alcohol, ethyl alcohol, and acetone to be more effective solvents than pure water.

Thermolability of Vegetable-tanned Leather

The general subject of the behavior of collagen and leather toward heat has been discussed in Chapter 5. The "boil test" has long been employed in empirically judging the degree of tannage of chrome leather, but it is only within recent years that the significance of a heat test in vegetable tanning has been recognized. By heat test is meant the behavior of leather when heated in water or other media, and the temperature at which the leather shrinks in area is termed the "shrinkage temperature" (sometimes designated "S. T.").

In 1924 Powarin and Aggeew[86] reported that, whereas calf skin showed a shrinkage temperature of 67°, this value increased to 84° after 32 days' tannage in quebracho liquor. Chater[21] has published a series of articles dealing with the effect of heat upon vegetable-tanned leathers. He secured samples of steer hide from different stages of the vegetable-tanning process: (a) after two days' tannage, (b) half tanned, and (c) fully tanned. The shrinkage temperatures were determined, and when the results on all specimens were averaged, the values were: (a) 56°, (b) 67°, and (c) 68°. He did not give the analysis of the leathers examined. Hobbs[51] has studied the shrinkage temperature of steer hide before and after tanning with five different tannages: quebracho, cutch, chestnut, sumac, and a blend consisting of equal

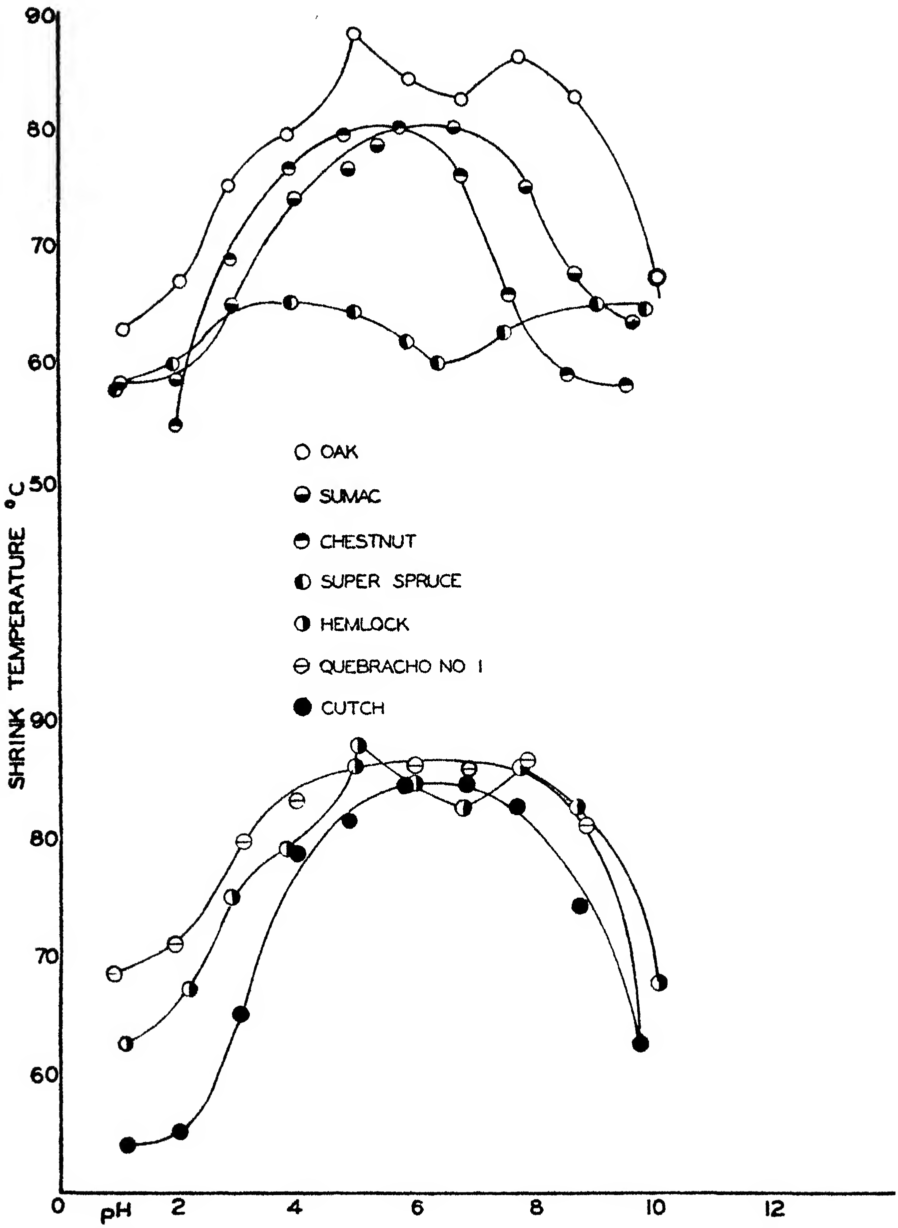


Figure 167. Shrinkage temperatures of some vegetable tanned leathers.

parts of chestnut, cutch, and sulfited quebracho. Time of tannage was up to 85 days, but no information is stated as to the liquor strengths, nor how the liquors were renewed; neither is the analysis of the leather given. The shrinkage temperature of the hide out of lime was 50° and out of bate, 60°; this was increased to a maximum which varied with the time of tannage and with the five different materials. The quebracho tan showed 86° after 40 days' tannage, cutch 85° at 70 days, chestnut 77° at 50 days, sumac 82° at 60 days, and the blend 79° at 20 days. Continued tannage caused these maximum shrinkage temperatures to drop. The maximum was regained, however, if the leather specimens were washed with water; in other words, as water-soluble material was removed. But in the absence of analyses of the specimens it is difficult to decide whether the rise of shrinkage temperature noted is a function of removal of uncombined matter or of that loosely combined.

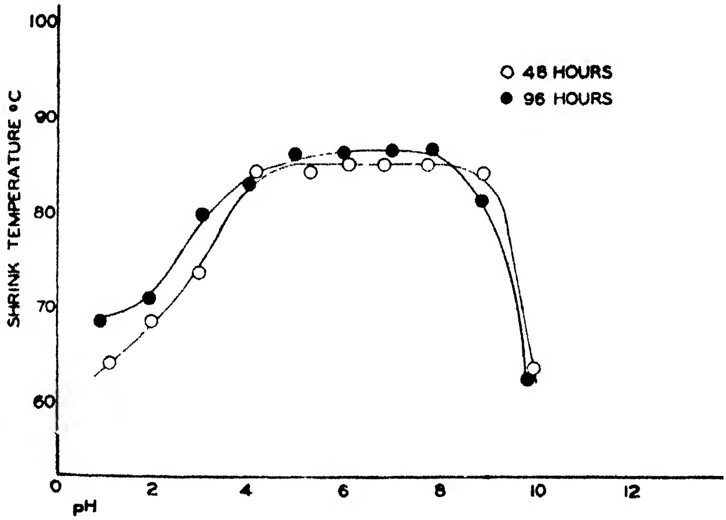


Figure 168. Effect of tanning period upon shrinkage temperature. (Quebracho tanned.)

Theis and Blum[107] have recently published extensive data on the shrinkage temperature of vegetable-tanned leather. They tanned goat skin for 48 hours at 20° in various commercial tanning materials, whose pH values were adjusted to range from 1.0 to 10.0, and whose tannin concentration was 1.0 per cent. After tannage the specimens were pressed in a hydraulic press to remove uncombined matter, and the shrinkage temperatures were then determined. The results are shown in Figure 167.

It will be noted that the shrinkage temperatures vary considerably with the different materials and with pH value and this indicates the importance of

pH values in vegetable tanning. The curves for both oak and hemlock show two distinct maximum values at approximately pH 5.0 and 8.0 respectively. Spruce extract, it will be noted, has but very little effect on shrinkage temperature over the entire pH range.

The effect of time of tannage is shown in Figure 168, where quebracho alone was employed, and in Figure 169, where a blend of chestnut and quebracho was used. These figures indicate that only in the more acid tanning ranges

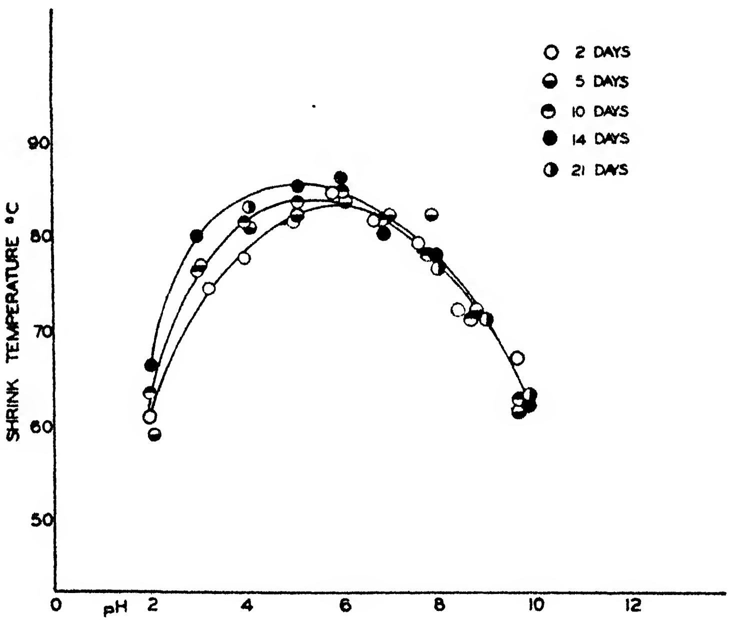


Figure 169. Effect of tanning period upon shrinkage temperature. (Vegetable blend.)

does any appreciable difference in shrinkage temperature occur as a function of tanning time, beyond a certain minimum.

Figure 170 illustrates the effect on shrinkage temperature when skin is tanned with quebracho alone, when it is pretanned with quebracho and retanned with 1.0 per cent formaldehyde for 24 hours, and when it is treated with a mixture of quebracho and formaldehyde. Both types of combination tannage raise the shrinkage temperature over that of straight quebracho tannage, elevating it in the pH range of 6.0 to 8.0 to above the boiling point of water. But when the combination tannage was reversed, that is, when the skin was first tanned with formaldehyde and then retanned with quebracho, a different result was obtained, as shown in Figure 171. Compared

with the reverse procedure (Figure 170), it is noted that a much smaller increase in shrinkage temperature was brought about by this type of retannage.

The results noted above were secured with freshly tanned and undried leather. Theis and Blum were interested in knowing whether the retannage effects shown would obtain if the vegetable leather was dried and allowed to

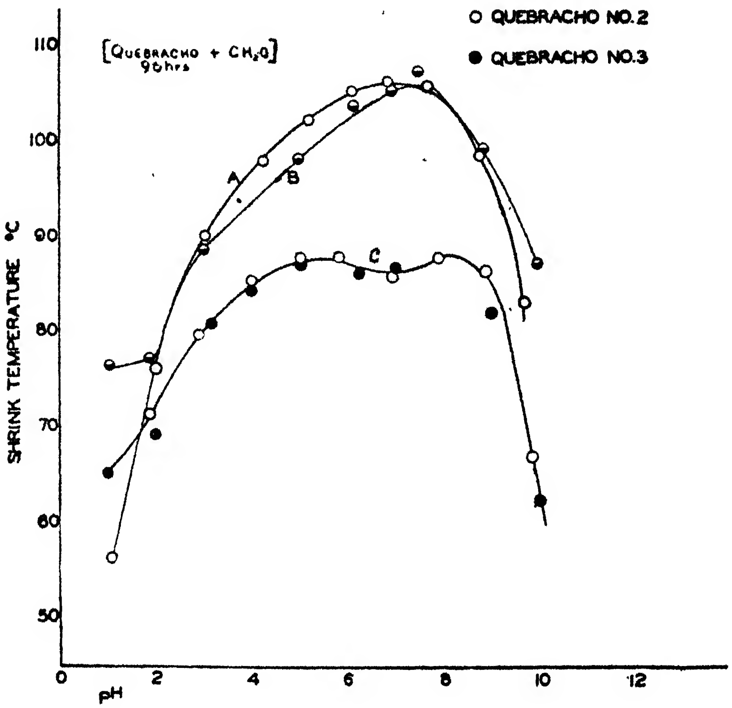


Figure 170. Effect upon shrinkage temperature of leather pretreated with quebracho and retanned with formaldehyde: "C" quebracho tanned only; "B" quebracho pretanned and formaldehyde retanned and "A" treated with a mixture of quebracho and formaldehyde.

age for several months before retanning with formaldehyde. Experiments of this nature were performed; they are shown in Figure 172 as Curve C. When the values of this curve are compared with those of Curve B, where the leather was brought to the pH values shown by means of acid or alkali, it is noted that shrinkage temperatures are greatly increased.

These authors comment upon their results as follows:

"There are at least two possible explanations for the effect of formaldehyde upon vegetable-tanned leather.

"The formaldehyde acts only as an independent tannage merely forming additional bonds or bridges between imino or amino groups of adjacent polypeptide chains. When formaldehyde is used previous to the vegetable retannage, certain of these reactive groups necessary for the vegetable-tanning reaction are preëmpted by the formaldehyde.

"The formaldehyde acts in conjunction with the vegetable-tanning material, causing certain polymerization of the anionic tannin molecules,

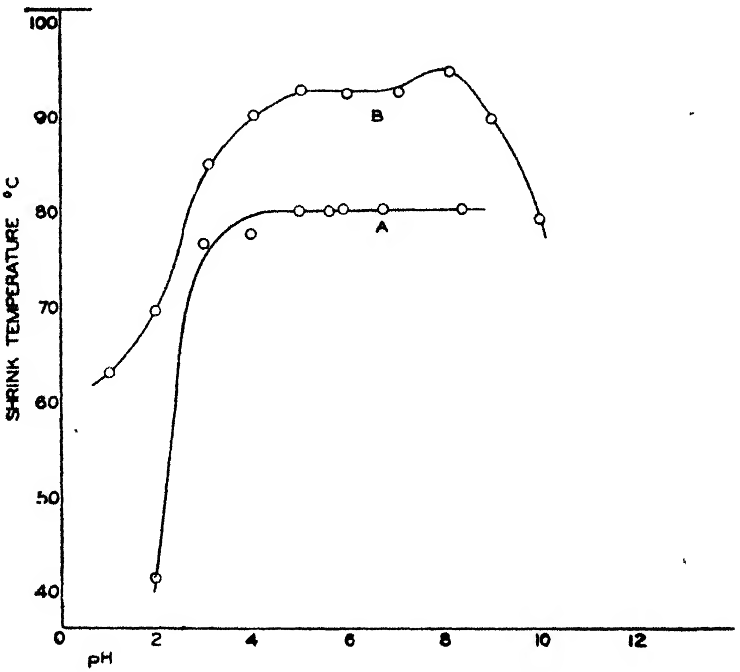


Figure 171. Effect upon shrinkage temperature of vegetable retannage of formaldehyde tanned leather, "A" formaldehyde tanned leather. "B" vegetable retanned leather.

giving increased size with subsequent greater tanning effect. It is a well known fact that formaldehyde reacts with certain of the vegetable-tanning materials.

"When formaldehyde is used previous to the vegetable extract, the formaldehyde has expended itself in forming its own particular type of bonds or bridges in the tanned skin and is not available for any action whatsoever upon the vegetable-tanning material."

In attempting to sum up this subject, we should point out that it is still

in its infancy but that the information already available indicates its importance. It is quite possible that shrinkage temperature measurements of vegetable leather, both finished and in process, may become of as great, or greater, importance as that of any of the older factors, such as pH value and combined tannin. Future shrinkage-temperature data would be made more

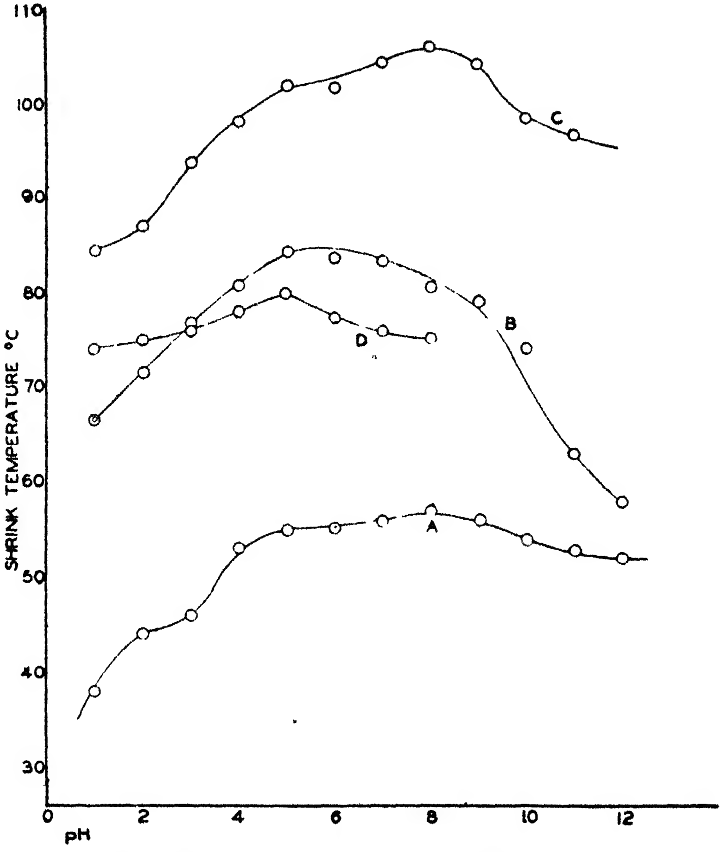


Figure 172. Effect of formaldehyde retannage upon commercial vegetable tanned leather. "A" shrinkage temperature raw skin over wide pH range. "B" effect of acid or alkali upon vegetable tanned leather. "C" effect of formaldehyde retannage of vegetable tanned leather. "D" effect of syntan upon vegetable tanned leather.

pertinent by the inclusion of the analysis of specimens, to include values of combined tannin and combined water-solubles. It would appear from the data above, that except in the lower acid ranges the amount of fixed or combined tannin—above a certain minimum—does not greatly affect the shrinkage temperature. For this reason it would be instructive to secure the

shrinkage temperature of leathers containing a wide range of combined tannin fixed from liquors of a common pH value. Each of the commoner tanning materials and blends thereof could be represented in the leathers tested.

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Chapter 18

Theory of Vegetable Tanning

There is as yet no inclusive theory of vegetable tanning; and it is very probable that no single theory will ever explain the many ramifications of the process. But the importance of understanding what occurs when tannin combines with skin to form leather cannot be overemphasized. In addition to the great theoretical interest and fascination of the reaction, we cannot expect to control the process in a scientific manner unless we understand its mechanism. Nor can we hope to synthesize the now greatly needed organic tanning materials successfully without understanding their real function. If the leather industry throughout the world is to meet the present and future challenge of substitutes, and the call for new types of leather, it can do so only through a sound scientific understanding of its materials and processes. The day of empiricism and pseudoscience in tanning has passed. Even though we still have much to learn of the tanning mechanism, a great deal of knowledge has been accumulated, as we have already noted in this volume. We shall now proceed to discuss the principal theoretical interpretations of the subject, although lack of space prevents complete consideration of the voluminous literature available.

In 1795 Seguin, a scientifically educated French tanner and a disciple of Lavoisier, explained tanning as a salt formation resulting from the reaction between basic hide substance and acidic tanning material. In 1803 Davy pointed out that the amount of tannin which combined with gelatin varied with the relative concentration of each. His general conclusion was that 85 parts of gall-nut tannin combined with 100 parts of collagen. In 1858 Knapp[11] suggested that tanning was a purely physical phenomenon, in that the tanning material merely coated the outside surfaces of the hide fibers, thereby rendering them immune to the action of dissolving agents as well as preventing their adhesion in drying. The work of these early investigators thus resulted in a sharp division of tanning theory into the "chemical" and the "physical." Such a strict distinction is no longer tenable, as we shall see.

Since many of the existing theories of vegetable tanning are more or less interwoven and often differ more in terminology and interpretation than in fact, we shall describe them as presented by their authors before discussing them.

In 1908, Stiasny[21] wrote: "Tanning consists primarily of the adsorption of the semi-colloidal substance which can undergo secondary changes leading to the irreversibility of the whole process." In the same year Wood stated: "An examination of the facts shows that the combination of gelatin and tannin compound is not of constant composition, nor a purely physical one, since it does not obey the solution laws, which require the concentration of the tannin in solution and the tannin in the gelatin to maintain a constant ratio." As a result of his studies of combination tannage with both chrome and vegetable tannin, Wood[30] wrote: "From this it will be seen that the tannin attaches itself to different bonds in the gelatin molecule from those to which the chromic oxide is attached."

In 1909, von Schröeder[26] suggested that vegetable tanning consisted of the neutralization of negatively charged tannin by positively charged collagen.

At about this period Meunier,[14] Fahrion,[5] and others suggested that vegetable tanning materials may form quinones on oxidation, and that some of these quinone bodies oxidize the amino groups of hide substance, which then combine with the quinones remaining in the tan liquor. Powarnin[18] suggested that quinone formation does not result from oxidation of tan liquors but is due to an isomeric change occurring in the tannin, whereby quinones are formed without the introduction of oxygen.

In 1921, Freudenberg[6] suggested that the combination between tannin and collagen is similar to that between a weak base, like aniline, with phenol, since Baeyer and Villiger found that many weak bases combine with phenolic substances in equimolecular proportions, or their multiples. And in 1934 Freudenberg[7] amplified his views as follows:

"The general property of all tannins is a high content of phenol groups in molecules which are more or less large. We shall now see that the interaction of tannins with the proteins of the skin is based on the reaction of phenols with amines and amides. Phenol itself, like many other phenols, combines with amines like aniline, or amides like urea, to form crystalline addition products. More complicated phenols show this property more distinctly, and tannins give precipitates with alkaloids, pyridine, or amides. More complicated amines and amides, *e.g.*, peptides, on the other hand, behave in the same manner. I am therefore of the opinion that the intermolecular forces, which allow phenols and nitrogen compounds to combine, are also responsible for the interaction of tannins and proteins. Opposite electric charges may, furthermore, contribute to the approach of the tannin particles to the protein particles, but the primary impulse will obviously be given by molecular forces."

In 1922, 1923, and 1924, Burgenberg de Jong[4] published articles of special importance which seem to have been largely ignored. He investigated the electrochemistry of pure tannin and found it to possess no electrical charge.

He pointed out that impure lyophilic colloids are generally precipitated by tannin regardless of whether they are negatively charged, as in the case of the colloidal carbohydrates, or positively charged, as in the case of skin proteins in tanning; and he suggested that the first reaction between tannins and hide substance is a rapid dehydration of the latter by the former. Subsequent chemical reactions between tannin and hide substance may occur, but the first and primary action is one of dehydration. The dehydrating power of a tannin increases rapidly with the number of phenolic groups in its molecule.

The fundamental concept of the dehydrating of hide substance by tannins has been quantitatively studied by Meunier and Le Vict[15] in 1929 and further discussed by Meunier[16] in 1930. These authors defined astringency as the ability of a tannin to contract tissues, and showed that this quality may be measured by changes induced in the plumping of hide substance. They defined water of plumping as water so held by hydrophilic groups of the protein that it cannot be removed by mechanical means, such as pressure or centrifuging. (In other words, they refer to "bound water.") Whenever these water-holding groups are transformed into a less hydrophilic state, tannage may be said to occur. Reduction in bound water of unhaired calf skin subjected to various tanning agents was determined. The skin was cut into cubes 3 mm on a side, and 3.0-gram specimens were centrifuged for 15 minutes at 3000 rpm and weighed; they were then tanned for 48 hours. After tanning, the specimens were washed free of soluble material and were then placed for 36 hours in a solution of acetic acid at a pH value of 2.4; after this they were again centrifuged and weighed, and then weighed again after drying. The difference in bound water of the tanned specimen compared with that of the control skin (similarly treated with acetic acid) is termed the "astringency value" of the tannin employed. When thus determined and calculated, quinone showed a value of 41.2, hydroquinone solution saturated with carbon dioxide 3.1, hydroquinone in the presence of light and air 31.0, gallotannic acid 23.0, gallic acid solution saturated with carbon dioxide 0.0, but in the presence of light and air 30, ordinary quebracho 29, sulfited quebracho 16, and formaldehyde 45. Meunier commented that the protein molecule is a complex construction of amino acids joined together by bonds of variable force, having free NH_2 groups at certain points. The water present in hide substance is either free or bound. The groups most active in holding the bound water are the NH_2, and the more of these that are present the greater will be the amount of bound water. But the protein molecule also contains many peptide linkages, which do not possess equal resistance to water and other disrupting influences, such as tannin. These linkages may open up and generate COOH and NH_2 groups—the latter immediately combining with the OH groups of tannin; and this splitting and combining

(tanning) process proceeds. This phenomenon is assisted by oxidation induced by the air which is occluded or dissolved in the tan liquors. Meunier ascribes the difference between slow and rapid vegetable tannages to the fact that in the former the bulk of the tannin of the leather is fixed or combined irreversibly, whereas in the latter it is combined reversibly.

In 1927, Li[12] studied a large variety of tanning compounds and pointed out that they all contained one or more OH groups. He advanced the theory that vegetable tanning consists of chemical combination between the NH_2 groups of hide substance and the OH groups of the tannin. He further suggested that the position of the OH group in the molecule of the tanning material is of great importance; that is, a single OH group must be near the center of the molecule, as in naphthol, or that two OH groups must occupy symmetrical positions near the extremities of the molecule. In other words, there must be a "balanced" molecular structure.

Lloyd[13] has interpreted tanning to mean suppression of the chemically active centers in the side chains of the collagen molecule, together with dehydration and protection of the peptide linkages of the molecular backbones. The carboxyl groups of collagen will be inactivated by the acid character of the tan liquor and the amino groups by chemical interaction with hydroxyl groups of the tanning agent. This chemical interaction is probably initially a salt formation and is later transformed into a covalent linkage; and in vegetable tanning, interaction also occurs with imino groups of the polypeptide molecular backbone. The strength of the chemical bond between collagen and tanning agent increases with the size of the tannin molecule. This is because of the balance of forces involved; valency bonds tend to hold together the interacting collagen and tannin, whereas the vibrational forces, due to the kinetic energy of the individual molecules, tend to shake them apart. As all molecules in equilibrium have the same kinetic energy, small molecules vibrate more violently than large ones. It follows, therefore, that if a collagen molecule is inactivated by a number of small tannin molecules, the balance of forces holding tannin and collagen together will be less than if all the active centers of the collagen molecule are inactivated by a single large tannin molecule.

Phillips[17] views vegetable tannage as the displacement of bound water from ionogenic groups of both collagen and tannin, and believes this occurs by mutual satisfaction of residual valencies. In other words, the residual valencies are exercised toward one another and not toward water molecules.

Braybrooks, McCandlish, and Atkin[3] have suggested two types of reaction between tannin and collagen: (1) An acid/base reaction between tannin and collagen amino groups, (2) condensation reactions of various types, which involve replacement of bound water by tannin. This second type is readily reversed by water and gives rise to "combined water-solubles." The first

reaction type is responsible for "combined tannin," and is dependent on pH value only above pH 8.0. Combination of tannin with amino groups is never complete, because these groups are never wholly accessible to the tannin. Accessibility is increased by the swelling of collagen, which explains increased tannin fixation as a function of swelling.

In a series of papers starting in 1936, Wilson[28] considered the mechanism of various tannages from the electronic standpoint. He concluded that effective vegetable tanning consists of the linking together by the tannin of the adjacent polypeptide chains at two or more points. In this way he explains the difference in the tanning properties of an ineffective tanning agent like lignin sulfonic acid and an effective agent like quebracho. By effectiveness is meant the resistance of leather to heat, as measured by its shrinkage temperature. Skin may be tanned with lignin sulfonic acid to show a very considerable amount of "combined tannin," but its shrinkage temperature is very low, whereas that of quebracho-tanned skin is very high. This is because quebracho, being polyfunctional, links together the polypeptide chains at two or more points, whereas the lignin sulfonic acid is unable to do so. In other words, the effectiveness of a vegetable-tanning agent cannot be measured by the degree to which it combines with collagen, but is determined by the kind of combination. Theis and Blum[23] have studied these phenomena at great length, employing many different vegetable tannins and reaching the same general results and conclusions as those of Wilson.

A number of workers have shown that, when hide powder is treated with increasing concentrations of tannin and the total absorbed tannin is plotted as ordinate and the unabsorbed tannin as abscissa, a parabolic curve results; and when the logarithms of such values are plotted, a straight line is obtained. These workers considered these results as indicative of an adsorption reaction. In the light of modern knowledge such results cannot be assumed to explain fundamentally, but only to describe the course of the reaction.

Stiasny[22] summarized the mechanism of tanning as follows: "Tanning means the transformation of the lyophilic groups in hide collagen into lyophobic groups. This transformation can be obtained either by reactions between active groups of the collagen and hydroxy groups of the tanning agent, due to secondary valencies on both components, the vegetable, mineral, and fat tannages being examples of these kinds of action; or by such reactions between hide and tanning agents, whereby primary valencies are concerned, not necessarily including the process of salt formation. Examples of this kind of tanning are given by the formaldehyde tannage, quinone tannage, and tannage by halogens." Stiasny has long emphasized the importance of particle size of tannins in their tanning behavior, and he was the first to attempt to determine the degree of dispersion of tan liquors.

In 1916, Procter and Wilson[19] stated their theory of vegetable tanning—

the outgrowth of their well-known application of the Donnan membrane equilibria to the swelling of gelatin. Their conceptions, briefly stated, were as follows. In normal tanning the tannin is negatively charged and the hide substance positively; thus when the two oppositely charged bodies meet, electrical neutralization and co-precipitation of the two colloids follows. They stated: "The rate of tanning will be a maximum for a given concentration of liquor when the potential differences are of opposite signs and the absolute value for each is a maximum. As the concentration of electrolytes in the solution is increased, the potential differences between the solution and both the jelly phase of the hide and the surface layer surrounding the tannin particles will decrease, lessening the rate of tanning; but if the concentration of the electrolytes is increased sufficiently, the tannin must precipitate alone and the collagen shrink to a hard mass. In alkaline solutions both colloids have negative charges, and consequently will not combine." This concept, it will be noted, is essentially the same in principle as that of von Schröeder. In a later publication Procter[20] stated: "We may thus divide vegetable tannage into two stages, in the first of which the tannins combine electrically or chemically with the fiber and render it insoluble, and in the second matters are deposited upon it which add to weight and solidity of the leather; but of course the two stages overlap in time and the different qualities of leather produced by different tannages are largely due to their relative proportion, and the amount of precipitable matter which the tanning materials contain." Wilson[27] has pointed out that the Procter-Wilson theory does not take into consideration the complex organic reactions which apparently occur in tanning with liquors which have pH values greater than 5.0 (the isoelectric point of collagen); nor is it concerned with fixation changes which may occur in the collagen/tannin compound in drying or aging.

Thomas[25] and his collaborators have made extensive studies of the application of the Procter-Wilson vegetable-tanning theory, and of the fixation of tannin by hide substance as a function of the pH value of tan liquors. The results of the latter studies are summarized by the general curve shown in Figure 165. Thomas has explained this curve as follows:

The rise in fixation from pH 5.0 (the isoelectric point of collagen) to pH 2.0 is readily understandable from the viewpoint of the Procter-Wilson theory, since in this pH range the collagen is positively charged and the tannin negatively. The fixation between pH 5.0 and 7.7 is a function of an intramolecular change of the collagen in this region, whereby the collagen is no longer entirely in its original state but has been partially converted into the "beta" form described on page 597. This second form of positively charged collagen is assumed to have an isoelectric point of approximately pH 7.7, is stable in alkaline solutions, and combines with negatively charged tannin. The decreasing but still appreciable tannin fixation between pH 7.7

and 11.0 is presumed to result from quinone-like bodies present in commercial tanning materials, since pure tannic acid showed practically no fixation in this pH region.

The difficulty in accepting the explanation given above for tannin fixations in the pH range 5.0 to 2.0 becomes apparent as soon as we consider the subject of electrical charges on tannin. When Thomas determined the electrical charge on various tannins (see Table 240), he unfortunately did not include pure tannic acid. But Bungenberg de Jong was unable to find any indication of an electrical charge on pure tannic acid. This immediately raises the important point that if a typical tanning material like tannic acid carries no electrical charge, we are not justified in assuming that tannage in the pH range 5.0 and 2.0 is principally a function of neutralization of electrical charges. The fact that commercial tannins do carry charges does not meet this objection. And even in the case of commercial tannin the evidence throws grave doubt upon the electrical charge concept, as the following considerations show. Thomas and Foster added hydrochloric acid to quebracho, and showed that when a pH value of 3.37 is reached the quebracho tannin is electrically neutral. But Wilson showed (Table 245) tremendous tannin fixations at pH 3.5, and general tannery experience confirms this. Again, Thomas and Foster showed (page 566) that at pH values below 2.0, hemlock, oak, wattle, sumac, and gambier all become positively charged, and yet their experiments (see Figure 165) indicated tremendous fixations at such pH values, where both collagen and tannin would be positively charged.

As to the tannin fixations between pH values of 5.0 to 7.7, which Thomas explains as a function of the conversion of collagen into the beta form, Atkin[1] claims to have proved that the second isoelectric point for gelatin or collagen postulated by Wilson cannot exist. This statement is made in an abstract of a paper entitled "The Titration of Gelatin and Collagen" delivered in 1937, but which we have been unable to find printed in full. There would seem to be little doubt, however, that the isoelectric point of unlimed collagen, or of that only slightly limed, is quite different from that of collagen which has been fully limed. In 1937, Gustavson[8] stated: "Unpublished determinations of the isoelectric points by the writer's chromium complex fixation method located the corresponding pH range as 7.0-7.5 for raw hide and an isoelectric point of the six days-limed hide in the range 5.5-6.0." In 1939, Highberger[10] electrophoretically determined the isoelectric point of collagen which had received only a mild alkaline treatment and found it to be at 7.8; and he suggested that the shift to pH 4.7 (the isoelectric point of commercial gelatin and of standard hide powder) is due to a fundamental change in the protein, caused by the alkaline treatment given these materials in liming. In the same year, Beek and Sookne,[2] employing essentially the same method, found a value of about pH 7.0. In 1940, Theis and Jacoby[24] secured a titra-

tion curve with Highberger's unlimed collagen, in the presence of 0.1N KCl, and found an isoelectric zone extending, approximately, between pH 7.0 and 9.0. The position of the isoelectric point of unlimed collagen, however, has not yet been found to bear upon the behavior of fully limed collagen with vegetable-tanning materials.

Page and Holland (Table 244) show essentially no difference in wattle-bark tannin fixation at pH values of 3.0, 5.0, and 8.0, and in Table 263 these authors show more combined tannin at a pH value of 8.8 than at 2.0. They are unable to explain the mechanism of tannin fixation at high alkalinities, which may be due to oxidation effects but, in view of the high tannin fixation values obtained, could hardly be due to quinone-like bodies. It should be noted that appreciable fixations in the alkaline ranges shown are contrary to tanning experience.

Gustavson[9] has recently suggested a mechanism of vegetable tanning; this publication is not available in the original but, in its abstracted form, is essentially as follows:

Vegetable tannin is fixed by collagen by means of two separate types of reaction, electrovalent and coordinate. In the first and most important reaction (whereby hydrothermal stability is attained), negatively charged astringent tannin combines with positively charged basic protein groups; this type of reaction occurs rapidly. The second, or coordinate reaction, is associated with the peptide groups, and is unaffected by the pH value of the tan liquor. Gustavson states that there is no sharp demarcation line between the two reactions, which proceed simultaneously. Gustavson's conceptions are essentially the same as those of Lloyd, described on p. 621. Both workers ascribe the leading role in vegetable tanning to an electrovalent reaction. But as we have pointed out, such a conception may not be profitably considered until we are sure that we are actually dealing with the electrovalent neutralization of oppositely charged bodies. If pure tannic acid carries no electrical charge, if quebracho is electrically neutral at a pH value of 3.37, and if it becomes positively charged at around pH 2.0, it would appear rather futile to ascribe a leading role to electrovalent reactions.

On page 614 we have discussed the mechanism of formaldehyde/vegetable tannage and have suggested the possible bridging of parallel protein chains by vegetable tannins by means of coordinate linkages between imino or amino groups in juxtaposition. It is quite possible that such a linkage is the important one in vegetable tanning and is the determining factor in the heat stability of the leather. This linkage would not, of course, account for all the tannin fixed by collagen; the additional fixed tannin may result from a coordinate linkage of the tannin bodies and the charged amino groups, without any bridging of protein chains. The first type of linkage seems to be very rapid, as we have noted, and is probably independent of pH value in the range

3.0 to 7.0. In this connection we may recall that Wilson and Kern tanned hide powder with quebracho at various pH values and then washed the tanned powder until only irreversibly fixed tannin remained; they found the amount of such fixed tannin to be constant in a pH range of 3.6 to 7.3. The second type of reaction probably accounts for the very necessary "filling" of the leather and for others of its properties, without contributing to its heat stability, as suggested by Theis and Blum[23] and by Wilson and Porth.[29]

The general conception of bridging or linking together of adjacent protein chains by tanning agents is one of importance in all considerations of the theory of tanning; it is a conception which is both logical and attractive. At the same time we must realize that we still lack final proof of its validity. Until such proof is obtained we can, however, employ it as a useful working tool.

In summarizing the various theories of vegetable tanning, we can see that there are many conflicting as well as common points between them. But with newer and better experimental methods at hand, the next decade should witness great advances in both theoretical knowledge and its practical application. We would now like to emphasize certain points which we feel to be of importance.

The employment of hide powder for experimental work is not to be generally recommended. When hide substance is finely divided by grinding, its physical condition becomes so different from that of the hide or skin employed in tanning that negative, or actually misleading experimental information, is often obtained. Pieces of hide or skin should be employed where possible. In order to have a uniform material, and one which will be uniform from lot to lot, we recommend for most purposes the material described on page 444.

The determination of bound water in tanning experiments is of importance. Very little information now exists regarding bound water values in tanning phenomena, but that little indicates the importance of such knowledge.

The subject of the electrochemistry of tannins is in a very unsatisfactory shape, as has been noted. If pure tannic acid carries no electrical charge, as Bungenberg de Jong states, or if commercial tannins are electrically neutral or positively charged at optimum tannin fixation pH values, many of our theoretical conceptions must be revised. Further information is needed on this whole subject.

Future tanning experiments may well include, together with fixed or combined tannin, determination of the combined water-solubles. This information will probably prove of increasing value in both theory and practice.

There now seems to be little doubt that the behavior of leather in the

presence of heat—the shrinkage test—is of fundamental importance in both the theory and practice of vegetable tanning. Further data in this important field should be gathered, and it should be correlated with leather composition.

The need of an adequate quantitative method for determining degree of dispersion of tan liquors has been stressed throughout the previous chapter. The fact that the attainment of such a method presents difficulty does not lessen its great importance.

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Chapter 19

Iron Tanning

The subject of iron tannage has long been of academic interest and thus a subject for scientific speculation and experimentation. The economics and universal availability of this particular metal have for centuries spurred the investigator in his effort to manufacture a commercially suitable iron-tanned leather.

Historical Development

An excellent summary of the history of iron tanning is contained in a treatise by Jettmar.[6] Johnson in 1770 obtained an English patent pertaining to the use of iron sulfate and hydrochloric acid as tanning agents. Some 24 years later another English patent was issued to Ashton. In this case, the ferric salt was prepared either by dissolving iron oxide in acetic acid or by oxidizing ferrous sulfate in one of a number of ways. In 1805, Hermbstadt investigated Ashton's patent and found that the best tannage was obtained by dissolving the iron in acetic acid. This is extremely interesting in view of our present view relative to organic salts of such metals as aluminum, chromium, and iron.

Knapp[8] obtained a number of patents covering iron tanning, but all his attempts to manufacture iron-tanned leather successfully failed; the leather was brittle and thin, and it deteriorated with age. Knapp mentioned that, while the tanning properties of ferric salts were well known, no one had as yet made serviceable leather from them. He recommended a method in which the skins were soaked in a solution of ferric, aluminum, or chromium salt and then in a soap solution. The fibers of the skin became coated with the insoluble soap of the heavy metal.

Jackson and Hou[5] made an extensive investigation of the various factors obtaining in the iron-tanning of sheep skins, including the best methods of oxidizing the ferrous to ferric salts and the relation of basicity to their stability and tanning properties. These investigators found that a slight excess of oxidizing agent before tanning is advantageous. They recommended the addition of a small quantity of oxidizing agent toward the end of the tanning process, since the skins and other organic matter present cause some reduction of the ferric to ferrous salts. They found the basicity factor to be very

important. Neutralization during tanning must be regulated so as to effect uniform fixation of iron throughout the skin and to keep the ratio of hydroxide groups to acid groups in the ferric salt between the values of 1:3 and 1:5. Before fatliquoring and coloring, the leather must be dried in order to cause maximum iron fixation and thus prevent interaction of the free iron salt with these added materials. Jackson and Hou pointed out that it was generally believed that the tanning salt had the formula $FeOHSO_4$ or was, in other words, 33⅓ per cent basic in character. They maintained that such a basic iron salt is unstable in solution, invariably giving a precipitate of hydrated ferric oxide. They claimed that the chief cause of brittleness of iron-tanned leathers is not the oxidizing action of the ferric salts but improper methods of tannage. In subsequent work, they prepared an iron-tanned leather which they were convinced compared favorably with other mineral-tanned leathers. It would not stand the standard shrinkage test, but had a shrinkage temperature in water of about 75°.

Procter[13] believed that the failure to produce satisfactory iron tannage might be due to the fact that iron salts act as oxygen carriers. His conception was that the ferric salt oxidized the organic part of the leather, becoming itself reduced to the ferrous state, slowly being re-oxidized by taking up oxygen from the air and then repeating this cycle. Casaburi[2] postulated that the poor results obtained in iron tannage were due to incomplete removal of uncombined ferric salts from the leather. He obtained a complete tannage with a 67 per cent acid ferric sulfate liquor, a less satisfactory result when ferric chloride was present, and poor results with normal ferric acetate. Casaburi followed his use of ferric acetate with that of other organic acids. He made quite extensive studies with citric and tartaric acids and claimed a satisfactory iron-tanned leather. His work, probably due to the patriotic motif "A Contribution to Italian Self-Sufficiency," is somewhat vague and clouded.

In 1928, Thomas and Kelly[15] approached the problem from an angle entirely different from any that had been used by other investigators in this field. They prepared a stock solution of pure ferric sulfate and made up their solutions for tests from this. They used only clear solutions which of necessity limited the range of concentration and basicity covered. Their procedure was to shake 2 grams of purified hide powder with 400 ml of the tanning liquor for definite periods, filter on Wilson-Kern extractors, wash free from soluble iron and sulfate, air-dry, and ash. In the light of present knowledge, this procedure would take into account irreversibly fixed iron only, and thus would not give the true picture of the iron fixation. The prolonged washing, which is quite contrary to actual tanning practice, would cause extensive hydrolysis of the ferric salt, remove a large portion of the fixed acid, and leave chiefly precipitated iron oxide.

Thomas and Kelly studied the basicity factor of the iron salt, effect of iron-salt concentration, effect of time and of neutral salts upon iron fixation. They found greater fixation of iron from the more dilute solutions than from the more concentrated ones, and also increased iron fixation with increasing basicity and the attainment of equilibrium at a fixed basicity and concentration in some 6 to 8 hours. With regard to neutral salts, these investigators found that sodium chloride decreased the iron fixation whereas sodium sulfate had very little effect. The complete studies of Thomas and Kelly are given in the second edition of this monograph and will not be further discussed here.

From 1928 to 1941 little attention was accorded the subject of iron tannage from either the theoretical or practical viewpoint. Late in 1941 the leather industry of North America was confronted with a shortage of chromium due the curtailment of chrome ore imports. As a result, several investigators began a serious study of iron tannage. The remainder of this chapter will be devoted to these recent studies.

Ruppenthal and Malik[14] attacked the problem of iron tannage from the viewpoint of the practical tanner. Their work is divided into two parts: iron tanning and combination iron-chrome tanning. They first investigated the use of ferric sulfate as a tanning agent and studied the potentialities of this reagent when used alone. They then proceeded to modify the ferric sulfate liquor by the addition of different sodium salts of various organic acids and various phosphates. They used the leather shrinkage temperature as their criterion of tannage. They found that iron phosphate-tanned skin gave a shrinkage temperature some 20° F higher than when iron sulfate was employed. They found organic acid salts to be beneficial, provided the acid contained a hydroxyl group in the molecule. As a result of these studies, they recommended the use of sodium gluconate along with sodium phosphate, since these two salts gave a leather having a shrinkage temperature of about 205° F. In subsequent studies, the ferric sulfate and chromic sulfate combination, together with the ferrous sulfate-sodium dichromate reaction, were extensively investigated. From their studies, Ruppenthal and Malik concluded that: (1) phosphate and gluconate should be incorporated in the iron-chrome tannage; (2) very little choice existed between the oxidation-reduction method and the ferric sulfate-chromic sulfate procedure, and (3) leather first treated with the iron liquor and then retanned with chromium salts gave the highest shrinkage temperature.

In 1943 Fleming[3] reported his investigation of iron tannage. Like Thomas and Kelly, he studied the effect of the basicity factor, of time and concentration, and the effect of neutral salts. Although repeating in some respects the work done earlier by Thomas and Kelly, Fleming made use of a certain analytical technic giving in some respects a somewhat different picture from that obtained by earlier investigators. In all his work, Fleming used 0.5-inch

bated calf skin squares prepared similarly to those used by McLaughlin and his associates. Samples containing 10 grams of hide substance, as calculated from nitrogen determinations, were first soaked 24 hours in 100-ml portions of distilled water at room temperature. The experiments were carried out in closed glass jars, with continuous agitation at room temperature. After the desired time periods of tannage, the samples were removed to funnels and allowed to drain, the liquors being collected in flasks for subsequent analysis. For his later experiments dealing with the ferrous sulfate-sodium dichromate reaction, the samples were washed after tannage and then pressed twice in a hydraulic press at a gauge pressure of 5000 pounds. In the experiments dealing with fixation of iron from ferric sulfate solutions, washing was eliminated, but a gauge pressure of some 22,000 pounds was used for pressing the samples.

Effect of Time and Concentration

These experiments were carried out by tanning pickled calf skin squares in tan liquors containing various concentrations of ferric sulfate over varying time periods, the overall basicity being maintained at $16\frac{2}{3}$ per cent. The sodium sulfate concentration was maintained at 5 per cent of the tan liquor volume. The results so obtained are shown in Table 265 and in Figure 173.

Table 265. Effect of Time and Concentration of Iron Fixation. From $\frac{1}{6}$ Basic Ferric Sulfate Solutions.

% Fe_2O_3 on Hide Substance	Grams Fe_2O_3 Fixed per 100 Grams Hide Substance			
	1 hour	3 hours	6 hours	24 hours
5	2.36	3.47	4 32	4.50
10	3.02	4 70	5.61	6.13
15	4 32		6.65	7.31
20	5.38	7 01	7.46	7.95
25	5.69	7.53	8.05	8.11

It can be seen from these data that: (1) equlibrium has been essentially established in some 6 hours and therefore a 24-hour tanning period is ample for establishing equilibrium under these set conditions; (2) with definite given basicity, the fixation of iron is proportional to the concentration of iron in the liquor; and (3) this proportionality is not general, since the iron is fixed to a much greater degree from the more dilute solutions.

Effect of Neutral Salts

In his early work, Fleming arbitrarily set the sodium sulfate content of his iron liquors at 5 per cent. Since sodium sulfate increases the pH value of ferric sulfate solutions and retards their precipitation, he investigated the effect of increasing concentrations of sodium sulfate on iron fixation. For these experiments, the salt content was varied between 4 and 10 per cent.

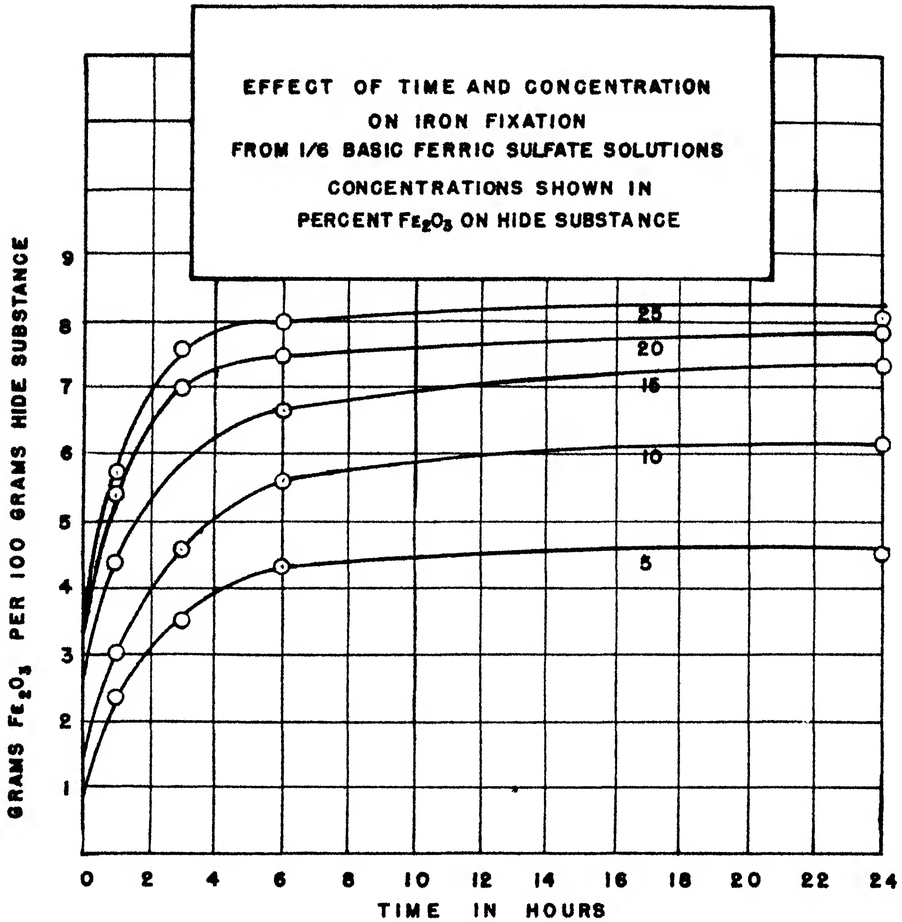

Figure 173

The procedure otherwise was that stated above. These data are given in Table 266 and in Figure 174.

Fleming points out that: (1) the effect of sodium sulfate on iron fixation is not great; (2) with increasing iron concentration, the effect of sodium sulfate

Table 266. Effect of Sodium Sulfate on Iron Fixation from $\frac{1}{6}$ Basic Ferric Sulfate Solutions

% Fe_2O_3 on Hide Substance	Grams Fe_2O_3 Fixed per 100 Grams Hide Substance 4% Na_2SO_4	6% Na_2SO_4	8% Na_2SO_4	10% Na_2SO_4
5	4.37	4.40	4.33	4.31
10	6 33	6.07	5.96	5.88
15	7.17	7.16	6.68	6.58
20	8.06	7.89	7.67	7.48
25	8.59	8.25	7.67	7.28

becomes more pronounced; and (3) that on going from 20 to 25 per cent Fe_2O_3, the effect of increasing concentration of sodium sulfate is such that less and less iron is fixed, until finally at 10 per cent concentration there is a maximum in the curve, showing less Fe_2O_3 fixed from the 25 per cent concentration than for the 20 per cent.

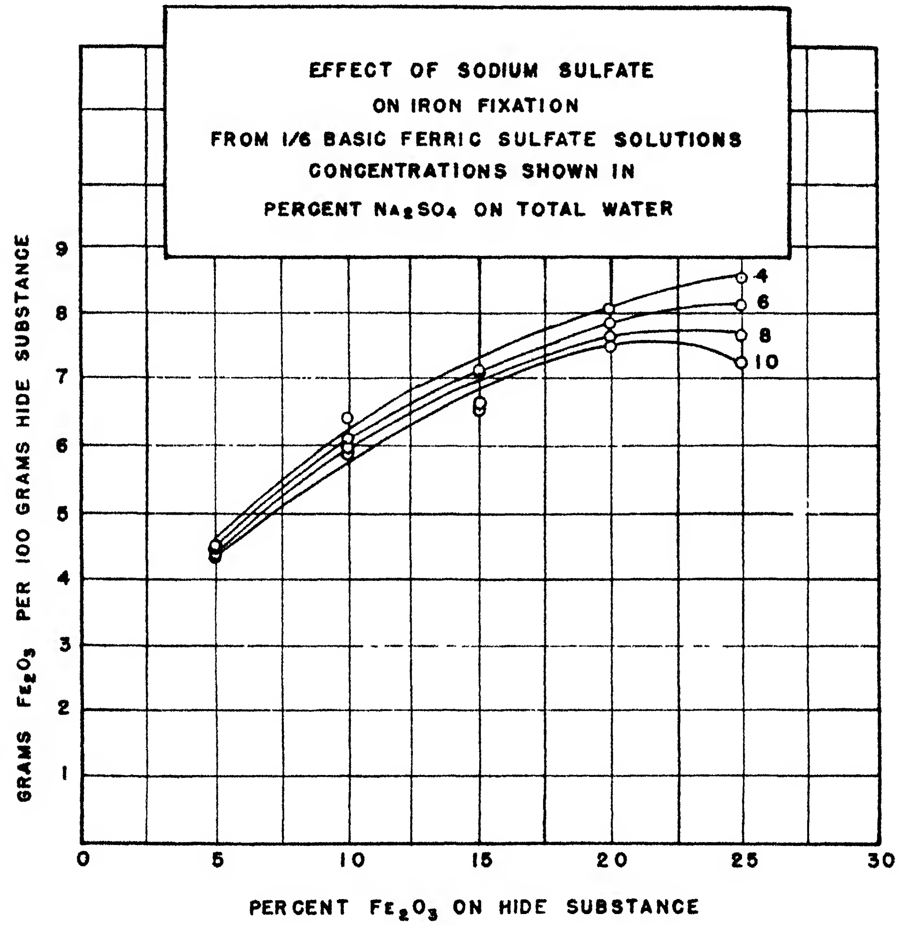

Figure 174

Effect of Basicity

Since it was shown that iron fixation by collagen is a function of its concentration at a given basicity, Fleming extended his studies to include other iron liquor basicities. The procedure was the same as that already outlined except for certain modifications made necessary by the range of basicities

employed. In order to retard hydrolysis and precipitation, the tan liquors were made up containing the requisite amounts of sodium sulfate and ferric sulfate and diluted to 50 ml. These solutions were then added to the pickled stock in the tanning jars and allowed to stand. After standing, the required quantities of sodium carbonate were added in 25 ml of solution and the whole mixed quickly. Tanning was for 24 hours after alkali addition. The analytical data obtained by Fleming are tabulated in Table 267 and are shown graphically in Figures 175 and 176.

Table 267

Original Concentration % Fe_2O_3 on Hide Substance	Grams Fe_2O_3 Fixed per 100 Grams Hide Substance = x/m	Grams Fe_2O_3 Unfixed per ml of Equilibrium Liquor = C	$C/x/m$	pH of Equilibrium Liquor	Condition of Equilibrium Liquor	Average Shrinkage Temp (°C)
8⅓% Basic						
5	3.96	0.00126	0.000318	2.60	clear	62 3
10	5.29	0.00567	0 001071	2 37	clear	65.5
15	5.72	0.01080	0.001890	2 27	clear	69.0
20	5.85	0 01662	0 00284	2 11	clear	71 5
25	5 88	0 0219	0.00372	2 00	clear	70 0
16⅔% Basic						
5	4.40*	0.00114	0 000240	2 62	clear	60 2
10	6.07*	0 00471	0.000752	2 39	clear	65.7
15	7.16*	0.00924	0 001293	2 30	clear	67.5
20	7.89*	0.01448	0 001831	2 23	clear	66.7
25	8.25*	0.01978	0.00238	2 19	clear	67 5
25% Basic						
5	4 38	0.00077	0.000175	2.70	clear	62 0
10	7 07	0.00381	0.000539	2.43	clear	65 0
15	8.26	0 00781	0 000946	2 33	sl ppt.	66 3
20	9 64	0 01231	0.001278	2.27	ppt.	69.5
25	10.13	0.01800	0 001774	2 22	ppt.	68.2
33⅓% Basic						
5	4.73	0.00063	0.000133	2 79	clear	60 3
10	7.19	0.00404	0.000562	2 50	ppt.	64.3
15	9.03	0.00755	0.000835	2 39	ppt.	65 8
20	10.38	0 01200	0 001157	2 31	ppt	68.3
25	10.57	0.01750	0.001654	2 27	ppt	67.3

* Average of two determinations.

Fleming states that his data indicate that the fixation of iron is an adsorption process dependent on the basicity of the ferric sulfate liquors used for the tannage. He further states that it is difficult to conceive of any adsorption reaction dependent on basicity other than that demonstrated by Cameron, McLaughlin and Adams for chrome tanning, in which they have shown that chromium deposition is dependent on the fixation of acid by the hide collagen, and therefore it is rational to postulate that the mechanism of iron tanning is similar to that of chrome tanning. Fleming predicates his assumption on

the following factors: (1) iron salts are similar to chromium salts in that they are readily hydrolyzable to free acid and a basic salt $Fe_2(SO_4)_3 + 2H_2O \rightleftarrows 2FeOHSO_4 + H_2SO_4$; (2) ferric hydroxide hydrosol is incapable of tanning and cannot penetrate the skin; (3) since it is known that iron tanning involves

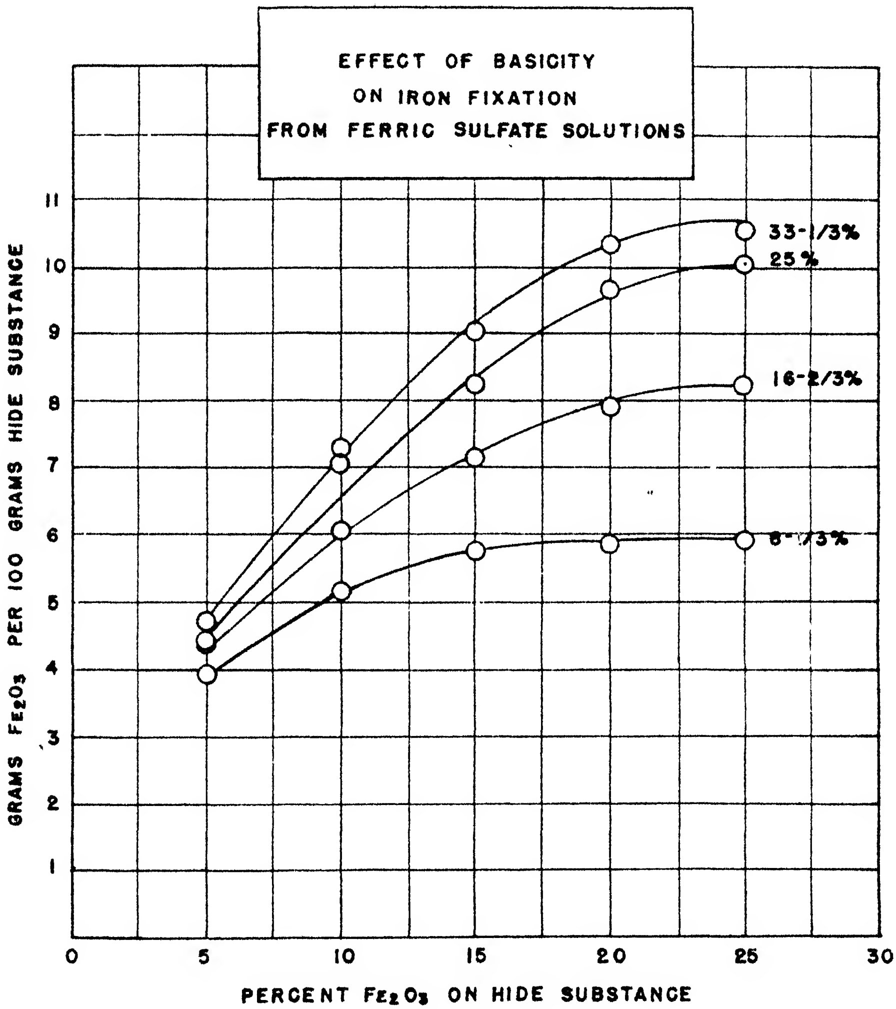

Figure 175

complete penetration, it cannot be assumed that precipitated iron salts are responsible for the adsorption-type phenomenon observed; (4) normal ferric sulfate or any other soluble compound, the concentration of which is not dependent on basicity and sodium sulfate concentration, cannot be the

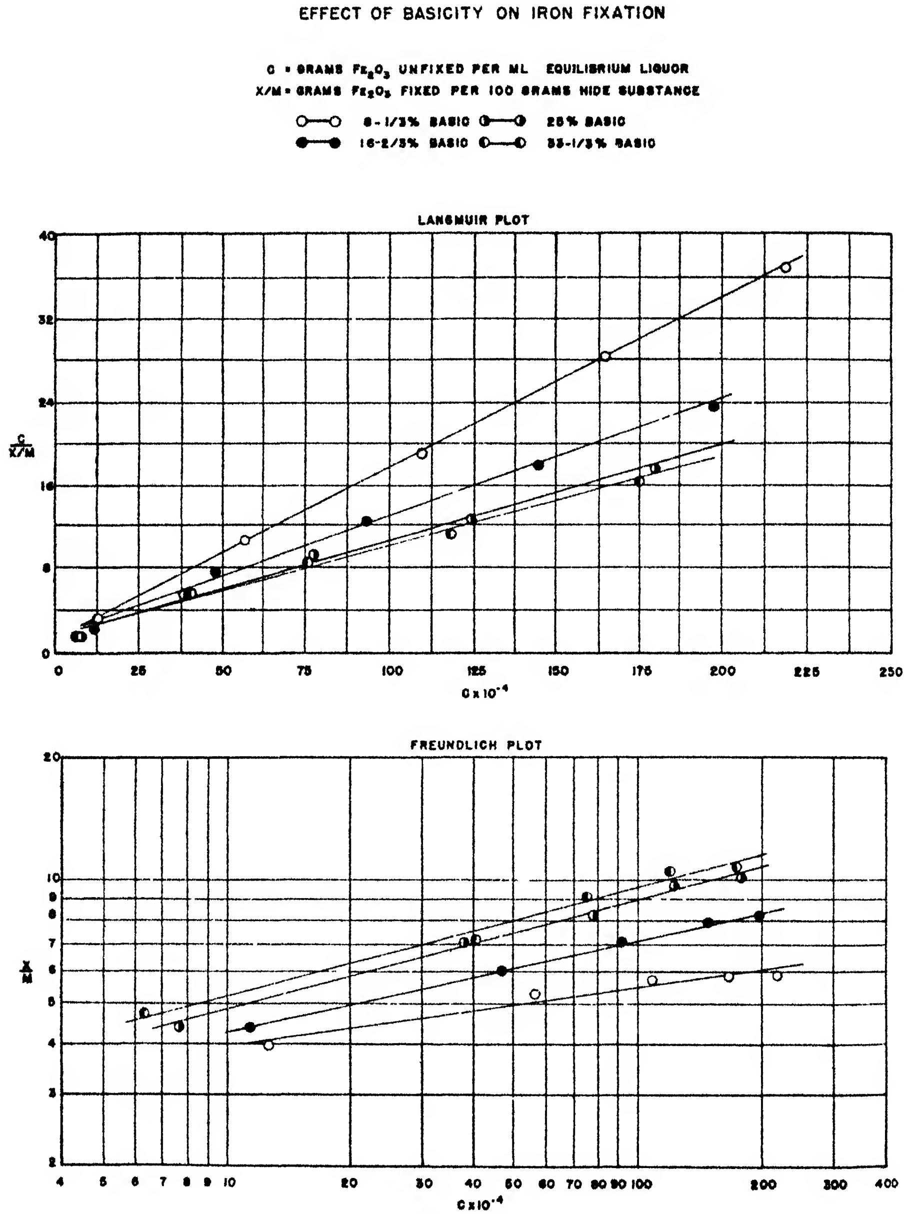
EFFECT OF BASICITY ON IRON FIXATION
C = GRAMS Fe_2O_3 UNFIXED PER ML EQUILIBRIUM LIQUOR
X/M = GRAMS Fe_2O_3 FIXED PER 100 GRAMS HIDE SUBSTANCE
8-1/3% BASIC
25% BASIC
16-2/3% BASIC
33-1/3% BASIC
LANGMUIR PLOT
C/(X/M)
C x 10^-4
FREUNDLICH PLOT
X/M
C x 10^-4

Figure 176

adsorbed material; and (5) it may be that the collagen fixed a specific basic ferric salt, since it is known that adsorption phenomena exhibit definite specificities.

Effect of Organic Acids

Kanagy and Kronstadt[7] investigated the effect on liquor stability and iron fixation of adding various organic acids to pure ferric sulfate. They used a reagent-grade ferric sulfate [$Fe_2(SO_4)_3 \cdot H_2O$] containing approximately 29.5 per cent Fe_2O_3 and about 56.6 per cent sulfate calculated as H_2SO_4. Tanning solutions were prepared by dissolving 40 grams of ferric sulfate and different amounts of the organic acid in 1000 ml of solution. One hundred-ml portions of such solutions were treated with varying amounts of sodium carbonate and made up to 200 ml. Table 268 shows the amounts of the different organic acids necessary to stabilize or prevent precipitation of the tanning solutions.

Table 268. Amounts of Organic Acids Required to Stabilize Iron-tanning Solutions at all pH Values in the Range 2 to 6

Acid	Grams acid per 40 g $Fe_2(SO_4)_3 \cdot H_2O$ in 1000 ml of Solution	Concentration (moles of acid)
Acetic	60.0	1.000
Lactic	9.0	0.100
Citric	6.4	0.033
Hydroxyacetic	15.2	0.200
Gluconic	19.6	0.100

The tanning properties of such solutions were determined by placing in them small pieces of steer hide. Tanning effects were determined by visual inspection and indicated a tanning zone in the pH range 2.5 to 4.5. These workers noted a slight tanning action throughout the pH range 1.75 to 5.5, but above or below these limits there was little fixation of iron.

Kanagy and Kronstadt studied the changes in the pH value of the various liquors upon aging. These data are given in Table 269. In general, the pH value of the solutions decreased on aging. They found a range of minimum stability for the solutions containing organic acids which appeared to be at about pH 3.0 to 4.5. These solutions appear to be stable at pH values greater than 4.5 or less than 3.0, a behavior which might indicate an amphoteric compound. Table 270 illustrates these observations.

The various tanning solutions were also studied by means of electrometric titration. For this investigation, 50-ml aliquots of solutions containing 40 grams of ferric sulfate, plus definite amounts of the organic acid in 1000 ml, were titrated with 1.0N sodium carbonate solution. The results are given in Figure 177; they compare data for each constituent used in preparing the tanning solutions. It would appear that the addition of the organic acid

Table 269. Changes in the pH Value of Iron-tanning Solutions with Time of Storage.

Iron-tanning solutions containing 8 g of ferric sulfate and different amounts of the organic acid in 100 ml were treated with varying amounts of sodium carbonate or sodium hydroxide and made up to 200 ml. The pH values of the solutions were determined immediately after preparation and after 24 and 48 hours.

Acid used	Concentration organic acid g 200 ml	pH measured immed	pH measured after 24 hours	pH measured after 48 hours	pH measured immed	pH measured after 24 hours	pH measured after 48 hours	pH measured immed	pH measured after 24 hours	pH measured after 48 hours
		1.2 gms Na_2CO_3			1.6 gms Na_2CO_3			2.0 gms Na_2CO_3		
Lactic	2.1	2.05	2.00	2.00	2.30	2.20	2.30	2.50	2.40	2.40
Lactic	4.2	1.85	1.80	1.80	1.95	1.95	1.95	2.15	2.15	2.15
Citric	1.3	1.85	1.75	1.75	2.15	2.15	2.10	2.40	2.40	2.35
Citric	2.6	1.50	1.45	1.50	1.60	1.60	1.65	1.80	1.80	1.85
Hydroxyacetic	4.3	1.90	1.80	1.85	2.00	1.95	1.95	2.35	2.10	2.10
Gluconic	3.6	1.75	1.75	1.75	1.95	1.90	1.90	2.10	2.10	2.05
		2.4 gms Na_2CO_3			2.8 gms Na_2CO_3			3.2 gms Na_2CO_3		
Lactic	2.1	2.70	2.40	2.35	2.65	2.40	2.40	2.70	2.45	2.45
Lactic	4.2	2.35	2.35	2.35	2.50	2.50	2.50	2.80	2.75	2.75
Citric	1.3	2.60	2.45	2.35	2.80	2.40	2.35	2.80	2.50	2.40
Citric	2.6	1.90	1.95	1.95	2.20	2.20	2.20	2.45	2.50	2.55
Hydroxyacetic	4.3	2.30	2.30	2.25	2.50	2.45	2.40	2.60	2.65	2.65
Gluconic	3.6	2.25	2.20	2.20	2.40	2.30	2.30	2.70	2.55	2.55
		3.6 gms Na_2CO_3			4.0 gms Na_2CO_3			4.4 gms Na_2CO_3		
Lactic	2.1	2.75	2.55	2.55	2.80	2.60	2.55	3.40	3.15	3.15
Lactic	4.2	3.05	2.85	2.80	3.20	2.95	2.90	3.45	3.10	3.15
Citric	1.3	2.85	2.60	2.55	3.05	2.75	2.70	3.40	3.10	3.05
Citric	2.6	2.75	2.75	2.75	2.85	2.80	2.75	3.70	3.25	3.20
Hydroxyacetic	4.3	2.70	2.70	2.65	2.95	3.00	2.90	3.80	3.25	3.15
Gluconic	3.6	3.15	2.75	2.70	3.35	3.00	2.95	3.70	3.30	3.30

Table 270. Ferric Hydroxide Precipitated from Iron-tanning Solutions.

40 g Ferric sulfate 0.05 mole gluconic acid and Na_2CO_3		40 g Ferric sulfate 0.075 mole gluconic acid and Na_2CO_3		40 g Ferric sulfate 0.075 mole gluconic acid and NaOH	
pH	g Fe_2O_3	pH	g Fe_2O_3	pH	g Fe_2O_3
1.5	0.00	1.5	0	1.7	0
2.7	0.04	2.6	0	2.5	0
3.3	1.04	2.8	0.48	3.4	0.84
4.6	2.40	4.0	1.24	4.0	0.06
5.8	1.16	5.7	0	5.3	0
6.3	0.26	6.0	0	10.3	0

increases the amount of highly ionized acid in solution, since increased concentration of these added acids causes the curves to approach that of the sulfuric acid. Citric acid appears to be more effective in this respect than lactic acid. Notice should be taken of the curve for the ferric sulfate alone,

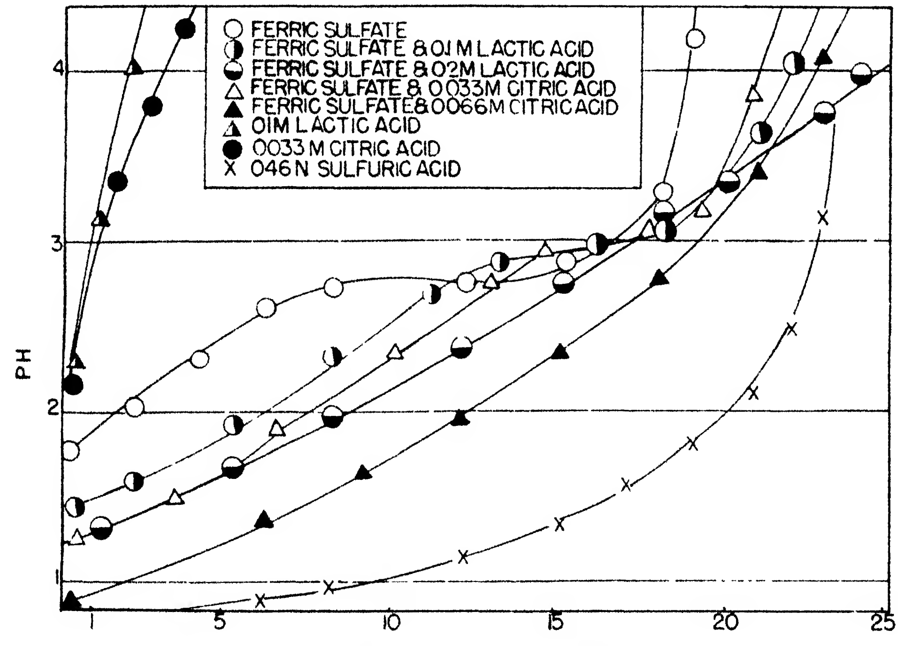

Figure 177

since it has a slope of zero at pH 2.7 to 2.8. In this region the iron is completely precipitated, but when the iron complex is masked by organic acid anions, precipitation does not occur.

Kanagy and Kronstadt suggest an explanation for the increase in titratable acid at pH values less than 3.0. They interpret this increase on the

basis of a change in the hydrolysis equilibrium of the ferric sulfate because of the formation of a complex ion between the organic acid and the iron, which they represent as follows in the case of lactic acid:

$$Fe_2(SO_4)_3 + 2H_2O \rightleftarrows 2FeOHSO_4 + H_2SO_4$$
$$FeOHSO_4 + HLa \rightleftarrows (FeLa)^{++}SO_4 + H_2O$$

These postulated reactions indicate the instability of the basic iron salt, which slowly hydrolyzes to form hydrated ferric oxide and is precipitated. On the other hand, the addition of an organic acid such as lactic acid causes a lactato-iron complex $(FeLa)^{++}$ to form, which is believed to be soluble. With the formation of this complex, the reaction indicated by the first equation will be driven further to the right, with the formation of more hydrogen ions.

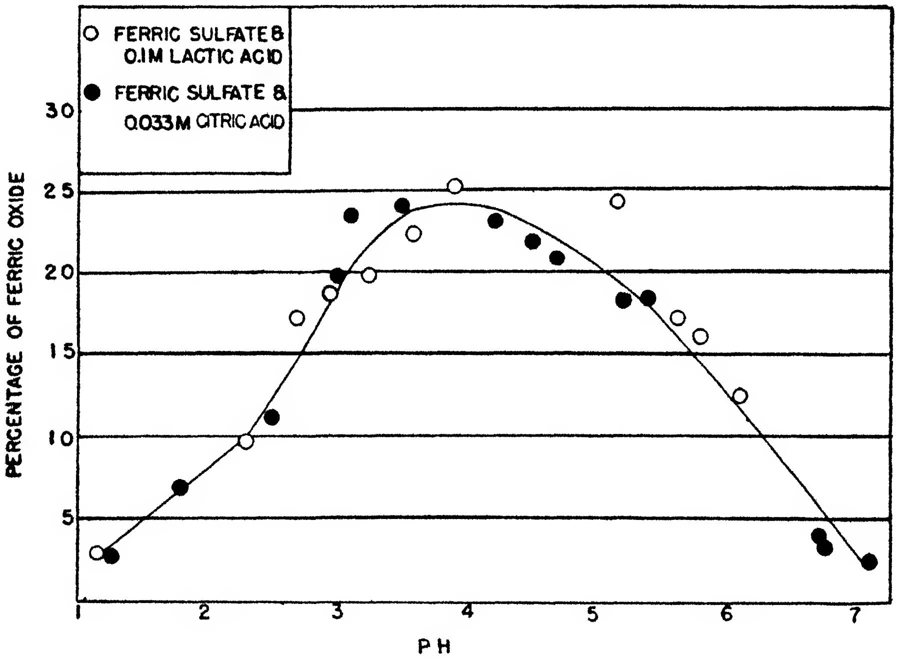

Figure 178

Minimum stability of the various tanning solutions used was noted in the pH zone 3.0 to 4.0, and Kanagy and Kronstadt explained this on the assumption that at pH values less than 4.0, the Fe^{+++} ion is cationic and at pH values greater than 4.0, the iron complex $(FeLa)^{++}$ becomes anionic, through reaction with sodium lactate to form $NaFe(La)_4$. The formation of the $Fe(La)_4^-$ complex at higher pH values decreases the tanning characteristics of the liquor.

Figure 178 shows the fixation of iron from the ferric sulfate-lactic acid

and the ferric sulfate-citric acid systems over the pH range 1.25 to 7.1. These results were obtained by shaking 4 grams of hide powder for 4 hours with 600 ml of solution containing 24 grams of ferric sulfate and 6.3 grams of lactic acid or 3.9 grams of citric acid. The pH value was varied by treatment with sodium carbonate. Maximum fixation obtained in the pH range 3.0 to 4.0, in which zone the basicity varied from approximately 0.66 at pH 3.0 to 1.0 at pH 4.0. At pH 1.0 to 7.0 only very small amounts of ferric oxide were bound. This is in line with expectation, since similar results have been obtained for chromic oxide fixation. In the very acid range, the skin is

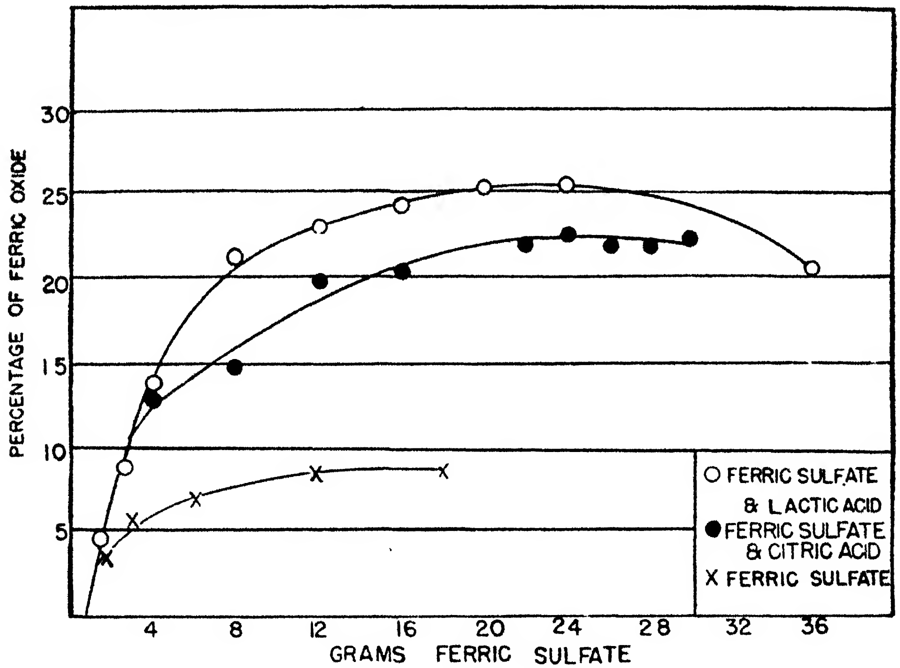

Figure 179

acid-saturated and, as shown by McLaughlin and Adams, is incapable of fixation of the chromic salt. As the pH value is increased, the skin proteins become less acid-saturated and are then capable of withdrawing acid from the tanning liquor, and the basic iron salt becomes fixed by the protein. The low fixation of iron at pH values greater than 5.0 is undoubtedly due to the formation of the nontanning anionic iron-lactate complex. Similar data have been obtained for anionic chrome tanning.

Kanagy and Kronstadt investigated the fixation of iron in relation to concentration. In this study, they compared the relative effects of ferric

sulfate alone, ferric sulfate plus lactic acid, and ferric sulfate plus citric acid. Their experimental formulation called for 4 to 36 grams of reagent-grade ferric sulfate in 600 ml of solution for each 4 grams of hide powder, for a 4-hour tanning period, and a hydrogen ion concentration of approximately pH 3.5 to 4.25. In the case of the lactic- and citric-acid systems, 1.06 grams and 0.64 grams for each 4 grams of ferric sulfate were used, respectively. Their data are given graphically in Figure 179. They definitely show that: (1) slightly higher iron fixation obtains with lactic acid than with citric acid; (2) higher values for iron fixation, in the presence of ferric sulfate alone, would have occurred if precipitation of hydrated ferric oxide had been prevented by masking.

Kanagy and Kronstadt also made certain practical tanning experiments. For this work they used pickled goat skins or calf skins. Approximately 300 grams of skin were drummed in 6 liters of a 5 per cent sodium chloride solution for 24 hours. To this was added 120 grams of ferric sulfate and definite amounts of the organic acid, as shown in Table 271. The necessary amounts of sodium carbonate were added to adjust the overall liquor to pH 2.5. After drumming for some 6 hours, additional sodium carbonate was added, and after the system had stood overnight the pH value was adjusted to 3.75-4.0, and drumming continued for an additional 6 hours. The skin was then washed, fatliquored, dried, sammied and staked.

The leather so obtained appeared to be of good quality. Its color varied from light yellow to brown but tended to darken on exposure to light. The physical and chemical analysis of the leathers are given in Table 271.

Table 271. Analysis of the Iron-tanned Leather and the Results of the Accelerated Aging Tests.

Skin No.	Organic Acid	Hide Substance (%)	Grease (%)	Ash (%)	Moisture (%)	pH	Shrinkage Temp (° C)	Original Tensile Strength (lbs/in²)	Loss in Tensile Strength on aging %
1	Citric	59 9	5.9	11 7	11.7	3.3	80	1850	20.5
2	Lactic	57.3	4.7	19.5	9.2	3.7	74	1640	37.2
4	Citric	58.5	1.0	15.4	9.6	3.9	84	2165	11.8
5	Citric	61.5	1.3	13 8	9.3	4 9	80	2925	19.7
10	Citric	58.2	7.7	13 4	7.0	3.4	76	2850	18 8
11	Citric	60.2	5.7	12.0	9.1	3.6	84	3620	27.8
12	Hydroxyacetic	54.1	9.0	15.4	7.9	3.6	75	3875	35.2
13	Citric	59.0	6.7	10.4	7.6	4.6	77	4895	31.3
15	Citric	47.7	21.3	10.1	7.1	4.5	80	4425	31 2
19	Gluconic	55.7	11.6	14.5	10.4	3.6	.	2905	50.1
20	Gluconic	55.5	12.7	7 6	10.7	3.4	77	3695	54.4
21	Gluconic	48.9	4.7	12.5	12.4	3.0	90	2980	55.2

Two-bath Iron Tannage

Several investigators have studied the reaction of the system: ferrous sulfate-sodium bichromate-sulfuric acid and its relation to iron-chrome tannage. Frey[4] mentions it in a patent obtained in 1930. Jackson and Hou[5]

studied its possibilities; and recently Ruppenthal and Malik[14] made use of this reaction in their practical studies. Fleming[3] in 1943 made a detailed and critical investigation of the reaction between ferrous sulfate and sodium bichromate. This reaction may be represented as follows:

$$6FeSO_4 + Na_2Cr_2O_7 + 7H_2SO_4 \longrightarrow 3Fe_2(SO_4)_3 + Cr_2(SO_4)_3 + Na_2SO_4 + 7H_2O.$$

The reaction occurs readily at room temperature and without development of heat and therefore lends itself nicely to a study of the two-bath method of iron-chrome tanning.

The Ferrous Sulfate-Sodium Bichromate System. Fleming first studied the absorption by calf skin of ferrous sulfate from a sulfuric acid and sodium chloride solution. Soaked calf skin squares, representing 10 grams of hide substance per sample, were agitated with 100-ml portions of solutions containing ferrous sulfate 2.5 to 15.0 grams, sulfuric acid 0.5 gram, and sodium chloride 15 grams. The tanning period varied from 1 to 24 hours. At the end of the desired period, the squares were removed, blotted lightly, air-dried, ground and analyzed. The data so obtained are given in Tables 272 and 273 and Figures 180 and 181.

Table 272. Absorption of Ferrous Sulfate by Calf skin from Solutions Containing 5.00 Grams H_2SO_4 and 150 Grams NaCl per Liter.

Grams $FeSO_4$ per liter	Grams $FeSO_4$ per 100 Grams Hide Substance 1 hour	3 hours	5 hours	24 hours
25	4.00	4.11	4 12	4 50
50	7.28	8 10	7.37	9.29
100	13.14	15 65	15.56	16.56
150	22.32	23.17	20.08	26.17

Table 273. Two-bath Method Using Ferrous Sulfate First, Control Series, 0 per cent Basicity.

(Average of two determinations)

Time (hours)	Grams Fe_2O_3 per 100 Grams Hide Substance	Grams Cr_2O_3 per 100 Grams Hide Substance	Ratio Fe_2O_3 Cr_2O_3
1	1.71	0.187	9.15
3	2.02	0.209	9.67
5	2.07	0.204	10.15
8	2.09	0.223	9.38
24	1.96	0.258	7.60

At low concentrations the ferrous sulfate absorption is practically complete in 4 to 6 hours but at higher concentrations absorption continues over a 24-hour period.

The skin treated with ferrous sulfate alone is of course untanned and therefore it must receive the second bath, of sodium bichromate and acid so that oxidation to the ferric state will take place. Fleming studied this reaction. Calf skin squares containing 10 grams of hide substance were

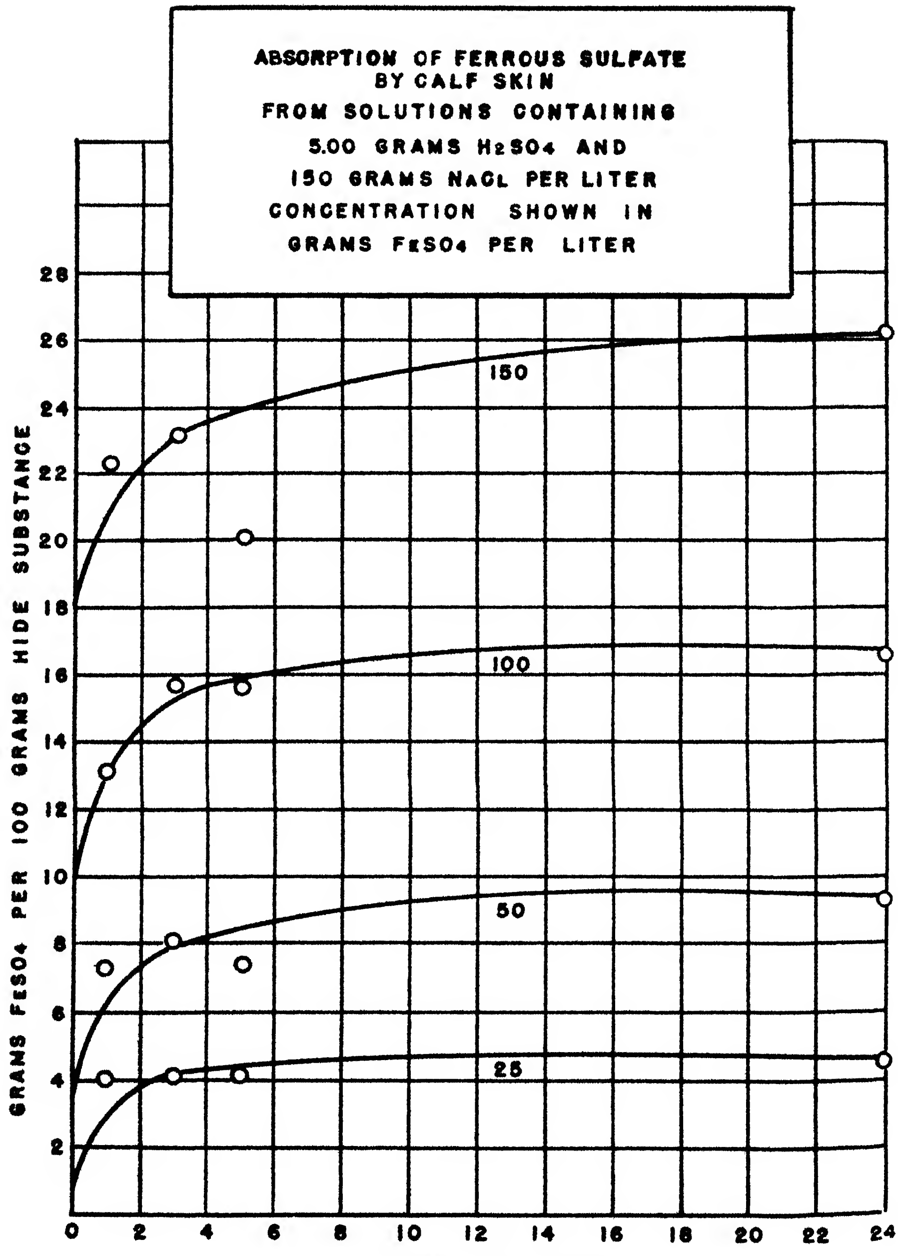
ABSORPTION OF FERROUS SULFATE
BY CALF SKIN
FROM SOLUTIONS CONTAINING
5.00 GRAMS H_2SO_4 AND
150 GRAMS NaCl PER LITER
CONCENTRATION SHOWN IN
GRAMS $FeSO_4$ PER LITER
150
100
50
25
GRAMS $FeSO_4$ PER 100 GRAMS HIDE SUBSTANCE
2
4
6
8
10
12
14
16
18
20
22
24
26
28
0
TIME IN HOURS

Figure 180

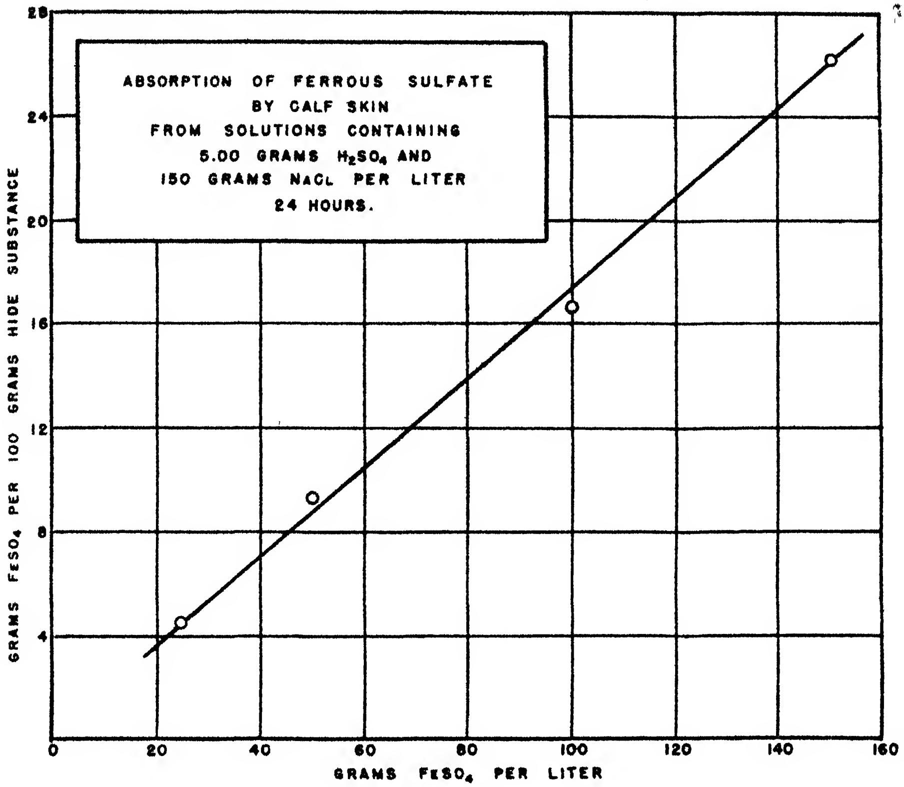

Figure 181

treated with 100-ml portions of solutions containing 7.5 grams of ferrous sulfate, 0.5 gram of sulfuric acid and 15 grams of sodium chloride for a 24-hour period. After this period, the squares were removed and were transferred to 50-ml portions of a second liquor containing 7.84 grams of $Na_2Cr_2O_7 \cdot 2H_2O$, 50 grams of NaCl and 15 grams of H_2SO_4 per liter. The oxidation-reduction period was 24 hours. However, at the end of 3 hours, the basicity was adjusted with sodium bicarbonate so as to obtain several different basicities. These data are given in Table 274 and Figure 182.

Table 274. Basicity Adjusted with Solution Containing 100 Grams Na_2CO_3 per Liter. Fixation at 24 Hours.

Ml Na_2CO_3 Solution Added	pH 30 Minutes After Na_2CO_3 Addition	Final pH	Grams Fe_2O_3 per 100 gms Hide Substance	Grams Cr_2O_3 per 100 gms Hide Substance	Ratio $Fe_2O_3 : Cr_2O_3$
2	2.00	. . .	2.86	0.347	8 24
4	2.31	1.90	3.88	0.458	8.43
6	2.62	2.05	5.00	0.522	9.58
8	3.21	2.05	5 92	0.943	6.28
10	3.70	2.30	6.24	1.080	5.78
12	4.51	2.96	6.47	1.400	4.62

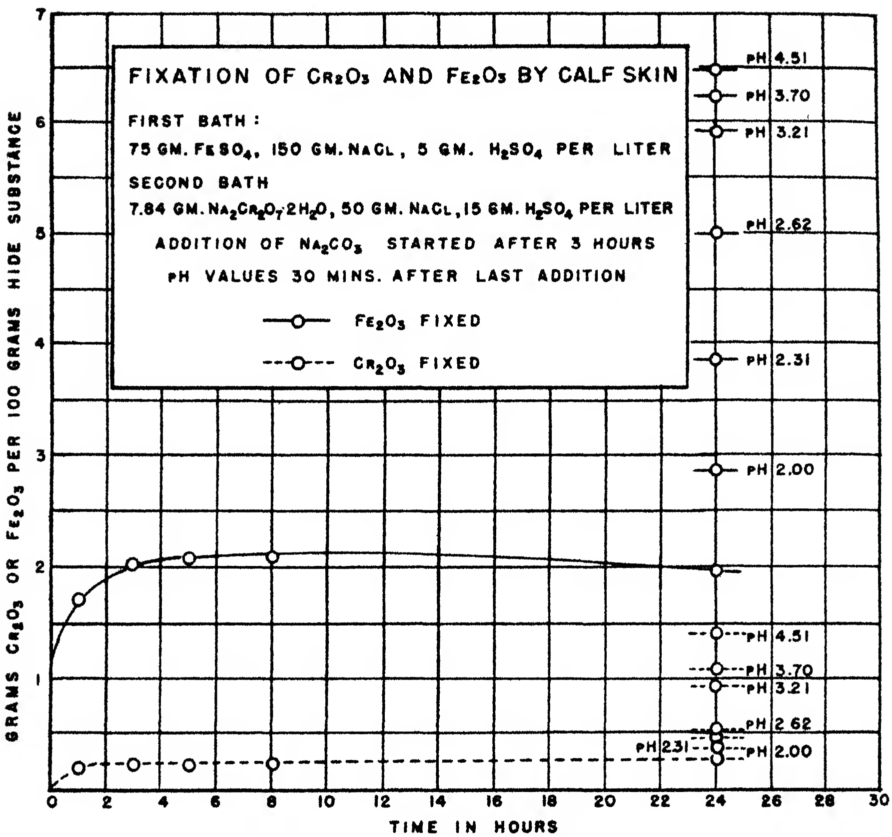

Figure 182

Just as in chrome tanning, very little actual fixation occurs until the overall basicity is increased.

The Sodium Bichromate-Ferrous Sulfate System. In this series of experiments, Fleming reversed his former procedure, using the sodium bichromate first and the ferrous sulfate second. He first studied the absorption of bichromate by calf skin; these data are shown in Table 275 and Figures 183 and 184.

Table 275. Absorption of Sodium Dichromate by Calf Skin from Solutions Containing 5.00 Grams H_2SO_4 per Liter.

Grams $Na_2CrO_7 \cdot 2H_2O$ per Liter	Grams Cr_2O_3 per 100 Grams Hide Substance 1 hour	3 hours	5 hours	24 hours
2.50	0.62	0.52	0.85	1.02
5.00	1.86	1.91	1.92	1.89
7.50	1.93	2.46	2.85	2.93
10.00	2.51	3.24	3.78	3.95

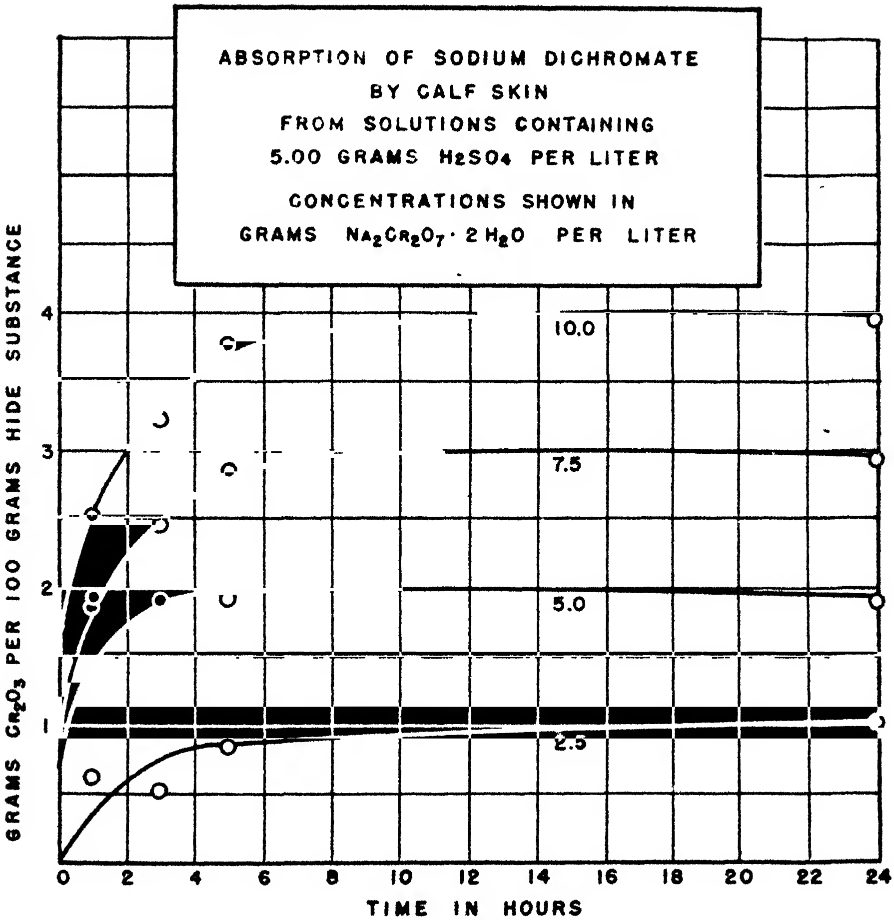
ABSORPTION OF SODIUM DICHROMATE
BY CALF SKIN
FROM SOLUTIONS CONTAINING
5.00 GRAMS H2SO4 PER LITER
CONCENTRATIONS SHOWN IN
GRAMS $Na_2Cr_2O_7 \cdot 2H_2O$ PER LITER
10.0
7.5
5.0
2.5
GRAMS Cr_2O_3 PER 100 GRAMS HIDE SUBSTANCE
1
2
3
4
0
2
4
6
8
10
12
14
16
18
20
22
24
TIME IN HOURS

Figure 183

In order to obtain 2 grams Cr_2O_3 absorbed, Fleming found it necessary to have a first bath consisting of 5.3 grams $Na_2Cr_2O_7 \cdot 2H_2O$ and 5 grams H_2SO_4 per liter. After treatment for 24 hours, the calf skin squares were

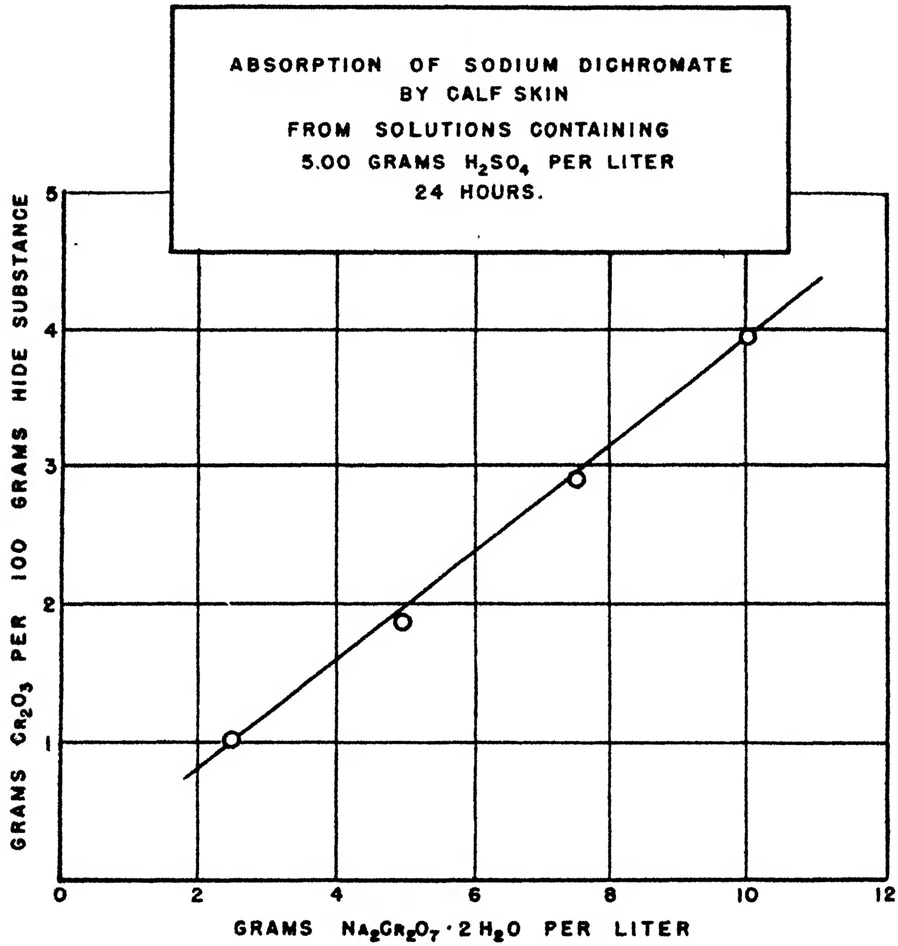

Figure 184

transferred to a second bath consisting of 50-ml portions of a solution containing 24 grams $FeSO_4$, 50 grams NaCl and 15 grams H_2SO_4 per liter. These data are shown in Tables 276 and 277 and in Figure 185.

Table 276. Two-bath Method Using Sodium Bichromate First, Control Series, 0 per cent Basicity.
(Averages of two determinations)

Time (hours)	Grams Fe_2O_3 per 100 Grams Hide Substance	Grams Cr_2O_3 per 100 Grams Hide Substance	Ratio $Fe_2O_3 : Cr_2O_3$
1	1.80	0.564	3.19
3	1.95	0.454	4.30
5	2.15	0.550	3.91
8	2.28	0.605	3.77
24	2.14	0.598	3.58

Table 277. Basicity Adjusted with Solution Containing 100 Grams Na_2CO_3 per Liter. Fixation at 24 Hours.

ml Na_2CO_3 Solution Added	pH 30 Minutes after Na_2CO_3 Addition	Final pH	Grams Fe_2O_3 per 100 Gms Hide Substance	Grams Cr_2O_3 per 100 Gms Hide Substance	Ratio $Fe_2O_3 : Cr_2O_3$
2	1.91	2.02	2.94	0.654	4.50
4	2.13	2.18	3.69	0.806	4.58
8	2.98	2.44	5.00	1.300	3.84
10	3.30	2.71	5.33	1.590	3.35
12	5.62	3.28	5.68	1.740	3.26

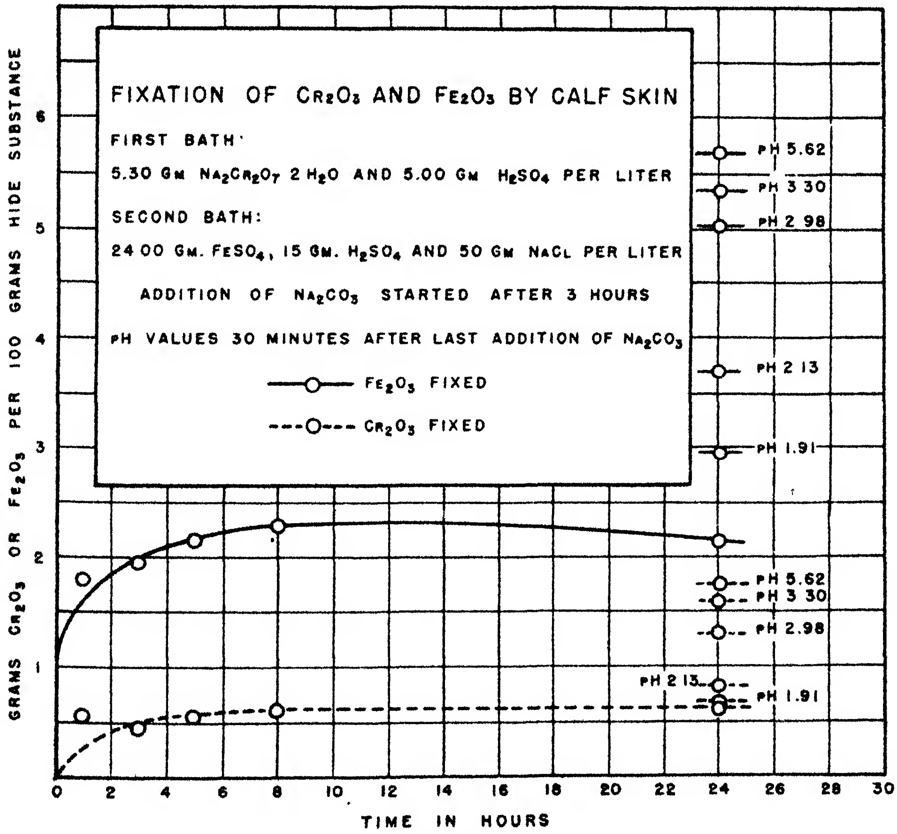

Figure 185

One-bath Iron Tannage

Sodium-Bichromate-Ferrous Sulfate System. Fleming studied a one-bath iron-chromium tannage by reducing the sodium dichromate with ferrous sulfate before contact with calf skin. He first pickled the calf skin pieces, using a pickle consisting of a solution of 5 grams of H_2SO_4 and 150 grams of NaCl per liter. A 24-hour pickling period was employed. The pickled skin was drained and placed in a one-bath tan liquor made up of 24.00 grams of $FeSO_4$, 7.84 grams of $Na_2Cr_2O_7 \cdot 2H_2O$, and 18.04 grams of H_2SO_4 in a liter. These reagents were allowed to react completely before use. Adjustment of basicity with sodium carbonate was the usual procedure. The data relating to this series of experiments are given in Tables 278 and 279 and Figure 186.

Table 278. One-bath Method, Control Series, 0 per cent Basicity.
(Averages of two determinations)

Time (hours)	Grams Fe_2O_3 per 100 Gms Hide Substance	Grams Cr_2O_3 per 100 Gms Hide Substance	Ratio $Fe_2O_3 : Cr_2O_3$
1	1.43	0.045	31.80
3	1.50	0.098	15.30
5	1.54	0.123	12.50
8	1.60	0.128	12.50
24	1.49	0.167	8.92

Table 279. Basicity Adjusted with Solution Containing 100 Grams Na_2CO_3 per Liter. Fixation at 24 Hours.

Ml Na_2CO_3 Solution Added	pH 30 Minutes after Na_2CO_3 Addition	Final pH	Grams Fe_2O_3 per 100 Grams Hide Substance	Grams Cr_2O_3 per 100 Grams Hide Substance	Ratio $Fe_2O_3 : Cr_2O_3$
2	1.80	1.87	2.16	0.282	7.66
4	2.18	2.12	3.03	0.355	8.54
6	2.30	2.22	3.97	0.478	8 31
8	2.52	2.37	4.73	0.788	6.00
10	2.74	2.51	5.47	1.060	5.16
12	3.61	2.82	6.23	1.340	4.65

Figure 187 graphically compares the data relating to the three different sodium bichromate-ferrous sulfate systems already discussed. This graph shows that when ferrous sulfate is used first there is a greater fixation of iron. It can also be seen that at the higher basicities, the one-bath method and the ferrous sulfate first two-bath method give about equal fixation of both iron and chromium, while the dichromate first two-bath method gives lower iron and higher chrome fixations. It appears rather evident that increasing basicity causes the iron to fix at a greater rate than chrome.

Fleming points out that if the ferric and chrome salts were fixed by the hide substance in exact ratio, as represented by the chemical equation, the ratio of Fe_2O_3 to Cr_2O_3 fixed in the leather would be 3 : 16. He found, however, that the actual ratio was always distinctly higher, due in all probability

to two factors. The first of these factors is the fact that iron salts are fixed more rapidly than those of chromium. A careful study of the data so far given shows that in the early stages of tanning the ratio may reach some 10 times the theoretical, thus definitely indicating the more rapid iron fixation. The second factor involved is the oxidation of the ferrous sulfate by air, which tends to increase the fixation of iron.

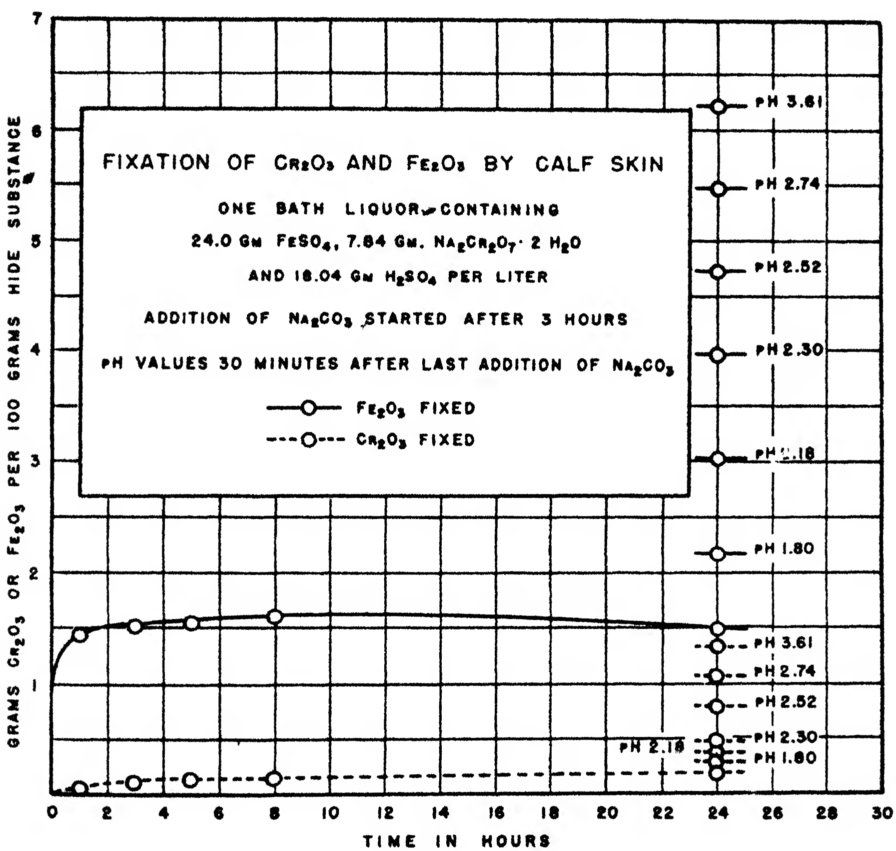

Figure 186

Theory of Iron Tanning

The published data show that ferric salts are tanning agents, whereas the ferrous salts are not, and that basic ferric salts are very much better tanning agents than normal salts. The best available data extant today definitely show that hydrated ferric oxide is not a tanning agent; therefore we are forced to consider the tanning action of the iron salts in a manner

somewhat similar to that of chromium salts. Cameron, McLaughlin and Adams[1] have suggested that the deposition of chrome is dependent on the adsorption of acid by hide substance and the deposition of $66\frac{2}{3}$ per cent basic chromium sulfate. Fleming suggests a similar mechanism for iron tanning, using the same reasoning.

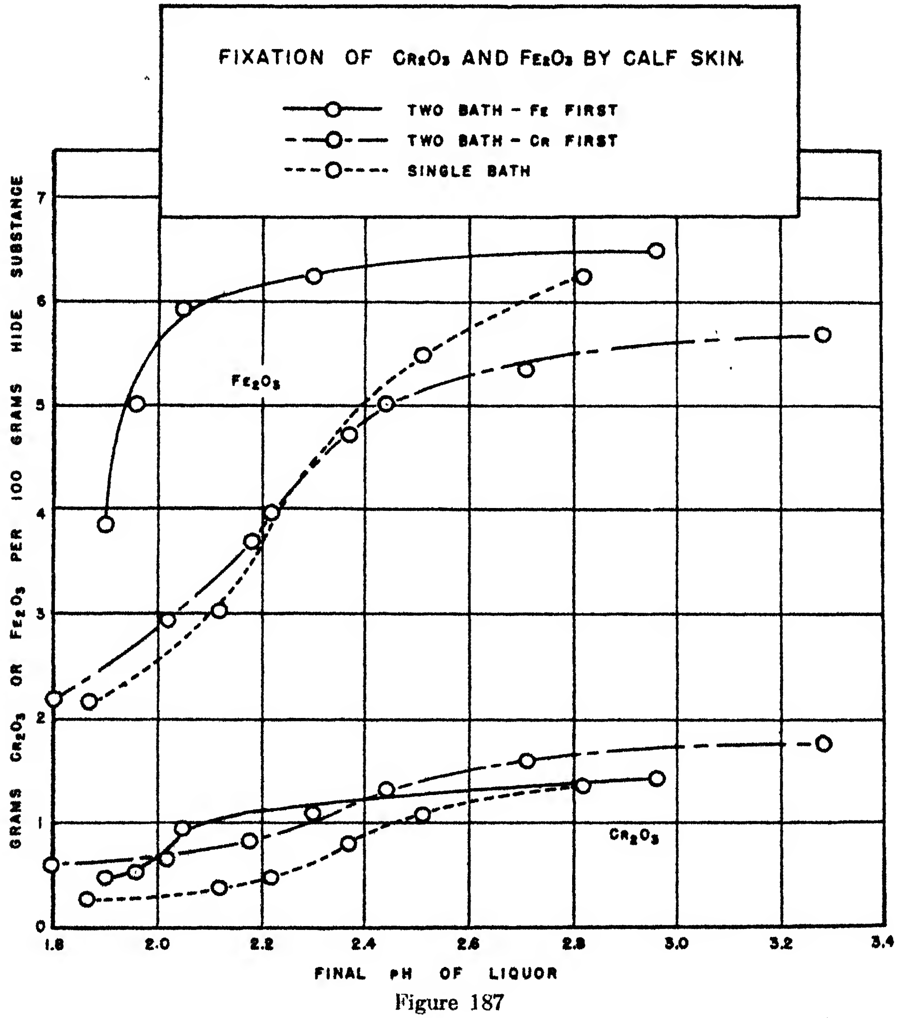

Figure 187

The fixation of the basic iron salt by hide substance appears to follow the same general principles as developed for the fixation of the chromium salts. As the pH value of tanning is increased, greater iron fixation obtains, until precipitation of hydrated ferric oxide occurs. If, on the other hand, ferric

sulfate is not used alone but is masked by some organic acid, such as lactic or citric acid, then iron fixation increases to a maximum value and then decreases, again following the same principle as for chrome tanning. The masking effects of the organic acids or their sodium salts bring about a less astringent tanning liquor and one containing both tanning and non-tanning iron complexes. We can visualize the various complexes from the following series of equations:

$$Fe_2(SO_4)_3 + 2H_2O \rightleftharpoons 2FeOHSO_4 + H_2SO_4$$
$$FeOHSO_4 + HLa \rightleftharpoons (FeLa)^{++}SO_4 + H_2O$$
$$2(FeLa)^{++}SO_4 + 2HLa \rightleftharpoons (Fe_2La_4)^{++}SO_4 + H_2SO_4$$
$$(Fe_4La_4)^{++}SO_4 + 2HLa \rightleftharpoons 2(FeLa_3)^{\circ} + H_2SO_4$$
$$(FeLa_3)^{\circ} + NaLa \rightleftharpoons (FeLa_4)^{-} Na$$
$$(FeLa_4)^{-} Na + NaLa \rightleftharpoons (FeLa_5)^{=} Na_2$$
$$(FeLa_5)^{=} Na_2 + NaLa \rightleftharpoons (FeLa_6)^{\equiv} Na_3$$

The tanning liquor can very well be made up of several of these ferric complexes; some have distinct tanning power while others are non-tanning in character. As the pH value of the iron liquor is increased, the equations are shifted to the right and the complexes become more anionic in character. At pH values less than 4.0, it would appear that the ferric ion is more cationic, while at pH values greater than 4.0 the ferric ion becomes more and more anionic in its character. Iron salts are not fixed by hide substance at either low or high pH values.

If the suggested mechanism of Cameron, McLaughlin and Adams is adopted, namely, that the fixation of iron is dependent on the adsorption of acid by hide substance, then the question of the nature of the iron compound arises. Certain investigators have denied the existence of basic ferric compounds and have regarded them as mixtures of solid solutions whose composition depends upon conditions of temperature and concentration at formation. Mellor[11] came to the conclusion that for straight hydrolysis of ferric sulfate solutions "so far as chemical analysis can tell, there might be an indefinitely large number of products where there is no break in the continuity of the process of transformation, by hydrolysis, from pure ferric sulfate to pure ferric hydroxide." Weiser[16] has shown that ferric hydroxide as such does not exist, but such compounds are various forms of ferric oxide with varying amounts of entrained water.

It is rather well known that ferric oxides adsorb considerable quantities of sulfate, the actual amounts of which are dependent on concentration, rate and other conditions of precipitation. Kolthoff and Sandell[9] have explained this condition: "It should be realized that from solutions having an acid reaction there will be primarily an anion adsorption. The surface of the hydrous oxide attracts the protons (H^+) and consequently an equivalent amount of anion is adsorbed. Thus, for example, it is found that sulfate,

chromate and oxalate are much more strongly adsorbed than chloride, nitrate, etc. In addition, the adsorption increases with increasing hydrogen ion concentration." Kraus[10] found that precipitates formed by the addition of alkalies, such as KOH, NaOH, NH_4OH, NH_2CO_3, and $NaHCO_3$, to ferric sulfate solutions are of variable Fe_2O_3/SO_3 ratio, depending upon the hydroxyl ion concentration and that a pH value of 7.7 must be reached before the precipitate becomes sulfate-free.

Fleming in discussing the type of iron salt adsorbed by hide substance states: "It should not be inferred that basic ferric sulfate compounds do not exist, as there is good evidence in the literature that such compounds do exist in solution and may be formed as stable solid products under certain conditions of Fe_2O_3 and SO_3 concentration. With regard to this, some experiments conducted by the writer have shown that there is a definite maximum at approximately 30 per cent basicity in the curve relating pH and basicity of ferric sulfate solution. This maximum could be interpreted to indicate that a basic compound is formed. However, examination of the conditions at which such basic ferric sulfates are formed as the stable solid phase (or precipitate) rather clearly indicates that they are not the conditions to be found in iron tanning, but are conditions of much greater Fe_2O_3 and SO_3 concentration."

Posnjak and Merwin[12] investigated the system $Fe_2O_3 \cdot SO_3 \cdot H_2O$ over the temperature range of 50° to 300°. They have constructed isotherms for each of the temperatures studied showing the solid phases in equilibrium with the saturated solutions over the entire possible range of concentration. Fleming states: "It is somewhat dangerous to try to extrapolate these data from their temperatures at 30°, the temperature of tanning. However, the data presented are such that it appears extremely unlikely that the rather dilute basic solutions used in tanning would have other than α-$Fe_2O_3 \cdot H_2O$ as the stable solid phase. This is especially true when it is considered that the hide substance adsorbs more acid from the system causing even higher basicities."

He further states: "In the above discussion it has been pointed out that the solid phase to be expected from solutions of ferric sulfate of concentration and basicity such as are used in tanning is probably α-$Fe_2O_3 \cdot H_2O$ or α-Fe_2O_3 with entrained water and adsorbed sulfate. It would seem very probably, therefore, that this is the type of compound which is fixed by the skin in iron tanning with solutions of basic ferric sulfate, unless, of course, the hide substance has a specific adsorption capacity for a certain type of soluble basic compound."

From such reasoning, Fleming suggests that the iron-tanning mechanism is the adsorption of acid from the basic ferric sulfate liquor by the hide protein, thus causing a deposition of the iron compound, which is probably ferric oxide, with varying amounts of occluded sulfate. This suggestion may not

obtain, if materials other than pure ferric sulfate, such as organic acids, phosphates, etc. are used.

Previous to 1942 no satisfactory iron-tanned leather had been produced, though a rather large volume of literature dealing with iron tannage was available. In 1942, due to a threatened chrome shortage, several satisfactory iron-chrome tanned leathers were commercially manufactured. Before 1942, the principal defects of an iron-tanned skin were such factors as brittleness, hardness, and instability to aging. Procter, Jackson and Hou and others have explained these seeming defects as being due to oxidation-reduction and to the fats and greases used in finishing operations.

What may be classed as a "good" or "poor" iron tannage is to some extent still an open question. The authors believe, however, that if proper attention is paid to the scientific principles of mineral tannage a satisfactory leather can be produced, especially if combination iron-chromium tannage is employed.

The whole question of iron tannage requires more study, especially with regard to the type, character, and stability of the iron complex and its fixation by skin proteins. Just as in chrome tannage, the masking effect of the organic acids will undoubtedly play an important role in subsequent work along this line.

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Chapter 20

Alum Tanning

The use of aluminum salts for the tanning of skins and furs was employed by the Romans some two thousand years ago and it was probably used by the Egyptians at a much earlier date. Though of ancient origin, this is not a true tanning process. The combination of collagen with aluminum does not take place nearly as readily as with chromium and the "leather" thus made is much less stable. Casaburi[3] states that the quantity of aluminum salts absorbed is not sufficient to produce a commercial leather. Since the skins are incompletely tanned, their stability toward the action of atmospheric humidity and toward water is poor and considerable hydrolysis of the aluminum salt absorbed within the fibers will take place, with formation of mineral acid. Because of this incomplete tannage, alum-tanned skins may be given a retannage with formaldehyde, a syntan or vegetable material. Because of the marked difference in tanning, the combination of collagen with alum is often called "tawing," to differentiate it from the more vigorous and stable tanning action of chromium salts and vegetable tannins.

The usual commercial method of alum tanning has been to place bated skins in a drum or paddle to which has been added potash alum, sodium alum, ammonium alum or aluminum sulfate plus sodium chloride. The skins are agitated in this solution for a number of hours and the liquor is then carefully neutralized with sodium bicarbonate. After neutralization, the skins are removed, allowed to drain for 24 hours, set out, shaved and then fatliquored.

The fatliquoring of alum-tanned skins has always been extremely unsatisfactory because such skins seem to repel the usual type of oil emulsions employed for the treatment of other tannages. In many cases, a paste composed of egg yolk, olive or neats foot oil and flour, is rubbed by hand into the flesh side of the skin. After this treatment, the skin is dried and allowed to age for some time, after which it must be wet, washed and again allowed to dry.

In the manufacture of white leathers, the alum-treated skin, before the fatliquoring operation or just after removal from the alum liquor, is placed in a formaldehyde solution at approximately pH 5.5 and given a retannage for about 2 hours. The skin so treated can now be easily fatliquored if the appropriate mixture of oils is employed. Another method is to treat the

alum-tanned skins with a rather strong solution of a syntan for about 4 hours. The syntan adds its effect to that of the alum and a more satisfactory commercial leather is obtained.

When aluminum sulfate is dissolved in water, hydrolysis takes place, giving rise to a soluble basic salt and an equivalent amount of free mineral acid. This reaction may be pictured:

$$Al_2(SO_4)_3 + 2H_2O \rightleftharpoons 2Al(OH)SO_4 + H_2SO_4$$

When a skin enters such a solution, the free mineral acid is taken up and fixed by the skin collagen in an amount depending on the pH value of the alum liquor. Swelling takes place just as in acid solution. As the free acid is fixed by the skin, a further quantity of the normal salt is hydrolyzed, giving more basic salt and free acid. As the acid is absorbed, the basic aluminum salt is also taken up and deposited upon and in the skin fibers, in a manner probably analogous to that taking place in iron liquors.

Skin tanned with an aluminum salt alone would be thin and hard and would be considered entirely unsatisfactory. However, if salt (sodium chloride or sulfate) is used in conjunction with the alum or aluminum sulfate an entirely different result is obtained. In the first place, the salt acts somewhat as in the acid-salt pickle, namely, it represses the acid swelling. In the second place, the salt allows a more basic aluminum salt to be formed, that is, it makes possible a higher pH value without precipitation. If the swelling action of the aluminum sulfate liquor were not controlled by the salt, little tanning would occur, since the tanning action of the alum is much too slow to prevent undue swelling.

Casaburi[3] points out that a chrome-tanned leather containing 2.5 per cent Cr_2O_3 is not resistant to boiling water and that a Cr_2O_3 content of 4.0 to 4.5 per cent is necessary. The corresponding amount of Al_2O_3 to impart boil resistance should be 5.0 per cent, and it would be necessary to add approximately some 50 per cent alum on the weight of the skins to produce this result. He further claims that the difficulty of obtaining absorption of such great quantities of alum by the skin fibers is mainly responsible for unsatisfactory alum-tanned leather.

The Aluminum Complex Ion

Küntzel[6] investigated the different behaviors of chromium and aluminum solutions. He reasoned that by studying compounds similar to those of trivalent chromium, light might be shed on chrome tanning. Aluminum resembles chromium in that it forms an insoluble colloidal hydroxide and complex ions in alum formation and in the crystalline structure of the hexaaquo chloride. When sodium hydroxide is added to chromium nitrate, the liquor remains clear until a given amount has been added, when cloudiness

results; with aluminum nitrate cloudiness results immediately and steadily increases with addition of alkali. Upon standing, this cloudiness disappears. Sodium hydroxide added to solutions of aluminum salts causes at first a rapid increase in pH value and then only a small, slow rise. The rise in pH value is less with aluminum than with chromium, indicating that the former has some kind of buffering action.

With chromium, stable complex ions are often found and are of extreme importance. Thus the carbonato complexes formed upon making chromium salts basic with carbonates hinder precipitation. In the case of aluminum salts, precipitation is inhibited but is of no real importance, since cloudiness soon develops and bubbles of carbon dioxide appear. The aluminum-carbonato complexes are considered to be very unstable, whereas those of chromium are of such stability as to resist prolonged heating. Similarly, the complex aluminum ions formed with many other anions are so unstable to alkalies as to render them impractical.

Neither aluminum nor iron salts given non-meltable gelatin gels. Aluminum does affect gelatin, however, increasing its viscosity and raising its melting point. Küntzel states that the solidification by tannage involves the approach or felting together of protein molecules, at first isolated, producing a coherent, porous structure. Each tanning chromium molecule must involve at least two protein molecules. The fact that aluminum reacts with gelatin without producing solidification indicates that the reaction must be fundamentally different from that of chromium. Küntzel believes that the behavior of chromium is not a characteristic sign of mineral tannage. He further points out that one should not apply the theory of chrome tannage to that of aluminum, as had been done by Wilson.

This investigator postulates that there is an essential difference between chromium and aluminum tannage, namely, the use of a rather large proportion of sodium chloride in the latter case, and that aluminum liquors need not be made basic. Pelt treated with aluminum salt only shows three characteristics: (1) great plumping occurs during the tannage; (2) alum-tanned skins dry horny and translucent; and (3) alum tannage alters the shrinkage temperature hardly at all. The effects of chromium salts are quite different. Alum tannage in conjunction with sodium chloride removes the first two characteristics but not the third. Küntzel believes that in alum tanning a pickling action is the essential principle, unless some other active component is present simultaneously. Alum tanning is not, however, just a pickling action, since some aluminum is fixed irreversibly.

Küntzel and Königfeld[5] in their first study of mineral tannage investigated the flocculation and cloudiness produced on making chromium and aluminum salt solutions basic. They found that these two salts behave quite differently on addition of alkali. Small amounts of alkali added to chromium chloride

solutions at minute intervals with constant stirring remain clear until a definite amount has been added. At this point the solution becomes cloudy, and thus has a definite precipitation point. With aluminum chloride or nitrate, there is a steady increase in cloudiness with no definite break, and one cannot speak of a real or characteristic precipitation point.

Küntzel and Königfeld found that if the intervals between alkali additions are still further increased to 24 hours, the cloudiness found after each addition has time to be redissolved at the lower basicities in the cases of the nitrate and chloride but that a sudden development of cloudiness is evident after a certain amount of alkali has been added and these liquors therefore show a precipitation point. The more concentrated liquors demonstrate this condition somewhat better. By this means a solution of aluminum sulfate (0.52 per cent aluminum) can be made 20 per cent basic without producing a permanent precipitate. If this solution is diluted, it will, after a short time, form a granular, crystalline precipitate which these investigators suggest may be the dihydroxo-tetraquo-aluminum salt

$$\left[Al\begin{matrix}(OH)_2\\(OH_2)_4\end{matrix}\right]^+_2 SO_4^{=}.$$

These investigators point out that it is an erroneous conclusion to relate the poor tanning action of aluminum sulfate to the difficulty of making its liquors basic; otherwise one would expect to tan with aluminum nitrate or chloride as well as with the chromium salts, since these can be made as basic as desired.

Upon making aluminum salt solutions basic, a flocculent, granular precipitate forms immediately. Küntzel and Königfeld suggest that the hydrolysis of an aluminum or chromium salt is different from that of KCN in that it does not involve external water and occurs as follows:

$$AlOH_2 \rightleftharpoons AlOH^- + H^+$$

and the hydrolysis constant is:

$$K = \frac{(AlOH)^- H^+}{AlOH_2} \quad \text{or} \quad K = \frac{(\text{basic salt})\ H^+}{\text{Hexaquo-salt}}$$

This type of hydrolysis is characteristic and involves the dissociation of a complex bound-water molecule. In the hydrolysis of aluminum and chromium salts, it proceeds in three stages and involves three constants. The real difference between aluminum and chromium lies in the fact that upon being made basic, the three constants are quite different for the two metals. In the case of chromium, the hydrolysis constant for the first stage is much greater than for the second or third stages, which are more or less equal. Thus on adding alkali to the hexaquo-salt, the first acid group must be

neutralized, forming the monohydroxo-salt, before the second and third groups are affected. This is indicated as:

$$\left[Cr(OH_2)_6\right]X_3 + NaOH \longrightarrow \left[Cr{OH \atop (OH_2)_5}\right]X_2 + NaX$$

In the corresponding aluminum salts, however, the three hydrolysis constants are approximately equal, which causes the immediate formation of aluminum hydroxide upon making the solution basic because of the locally higher pH value at the location of the drop; or, in other words, a complete neutralization of the three aluminum aquo acid groups occurs immediately. This may be expressed:

$$Al(OH_2)_6X_3 + 3NaOH \longrightarrow \left[\begin{matrix} OH \\ AlOH(OH_2)_3 \\ OH \end{matrix}\right] + 3NaX + 3H_2O$$

More specifically, it follows that in a chromium solution at the pH value corresponding to 33⅓ per cent basicity, essentially only the monohydroxo-salt is present, although the dihydroxo-salt may be present to a very slight extent. In the corresponding aluminum salt, the hydroxide is stable at the same pH value. Chromium salts are more hydrolyzed than aluminum salts in the first stage, so that the chromium solutions are more acid. However, the acid formed by hydrolysis of the aluminum salts is adsorbed more avidly by the hide proteins because of the weaker tanning action of the basic aluminum salts.

Küntzel, Riess and Königfeld[7] in a subsequent investigation relative to mineral tannage studied the aging phenomena occurring in basic aluminum and chromium solutions. They state that the behavior of aluminum and chromium salts in aqueous solution on addition of alkali is governed by the hydrolytic decomposition to free acid and basic salt and which can be termed primary hydrolysis; this is an ionic reaction, the equilibrium being attained quickly. A second factor enters, namely, the nature and manner of the aggregation of the basic salts from this primary hydrolysis to larger molecules and the conversion of these to acid-resistant forms. Such processes are termed aging.

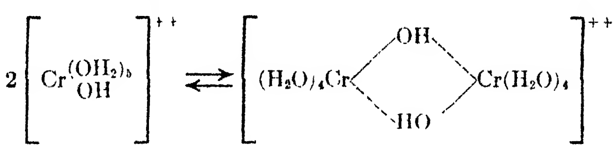
The aging changes are accompanied by a gradual increase in the acidity of the solution. This secondary hydrolysis occurs because the basic salts, formed by primary hydrolysis, aggregate and as a result tend to become insensitive to acids and are thereby removed from the hydrolysis equilibrium. The slow hydrolysis can best be followed by titration with alkali and hydrogen ion concentration measurements.

On titrating aluminum nitrate solution, the pH value rises rapidly until the basicity reaches 16 per cent and then remains essentially constant. On the other hand, titration of chromium nitrate shows a steady rise in pH value. Küntzel, Riess and Königfeld suggest that these results indicate that chro-

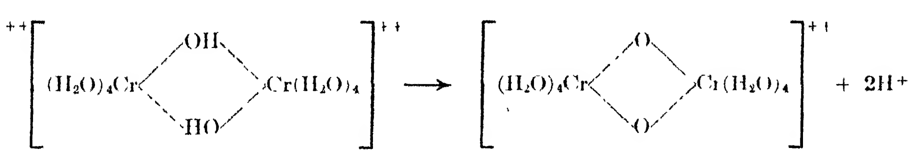
mium nitrate is initially more hydrolyzed than the aluminum nitrate and that the first stage of the hydrolysis of chromium nitrate is much stronger than the second and third stages, whereas with aluminum nitrate the three hydrolysis constants are approximately equal.

It has been noted that chromium and aluminum hydroxides become less sensitive to acids on aging, this effect being more marked with chromium. Aging involves the splitting off of water and the formation of large molecules, but there is apparently no relation between the size of the molecules and the resistance to acids. These investigators found in the course of their studies that chromium and aluminum solutions contain, at the same time, molecules of various sizes, and that the aluminum particles are smaller than the corresponding chromium particles. They maintain, however, that the difference in tanning characteristics cannot be attributed to this difference in molecular aggregation.

Stiasny[9] explains these secondary alterations or aging phenomena by means of his olation theory which may be expressed as follows:



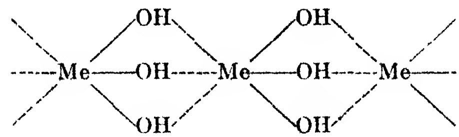
Thomas and von Wickeln[11] extended Stiasny's theory to include yet another reaction:



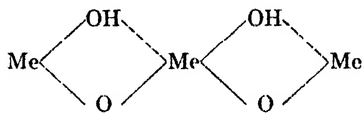
and in this manner explained the increase in acidity obtained through boiling the solution. This reaction was termed oxolation. Thomas has applied a similar reasoning to explain the formation of polymerized compounds in basic aluminum salt solutions. Jander[4] suggested the combination of several metal atoms by means of an oxygen bridge. He pictured the reaction as follows:

$$2 > \mathrm{AlOH} \longrightarrow > \mathrm{Al{-}O{-}Al} < + \mathrm{H_2O}$$

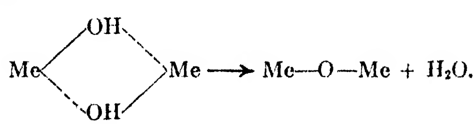
Jander[4] did not take into account Stiasny's theory of olation because he was primarily interested in the reactions of iron and aluminum hydroxides. He postulated the formation of meta-hydroxides (FeO·OH and AlO·OH) rather than the ol- compounds formed by chromium. According to the olation theory, one might expect as the end product:



Jander pictured for the iron and aluminum compounds the meta-hydroxides, which may be formulated:



Jander further suggested that chromium also gives a meta-hydroxide on aging; he pointed out that certain facts support the oxygen-bridge theory for iron and aluminum, and the hydroxyl-bridge theory for chromium, and that the adoption of one theory is important, since Stiasny's theory postulates that the ol- groups are concerned in the linkage of the chromium and hide substance. As to the actual facts, it appears easier to apply Jander's theory to chromium than Stiasny's to aluminum. It may very well be that aggregation is olation and that aging may involve the change of ol- compounds to oxo- compounds, as



With aluminum, these changes appear to follow each other with great rapidity and cannot be separated, while with chromium the changes are slow enough to be recognized as separate reactions.

Perkins and Thomas[8] in 1937 made a study of the olation of basic chromic, aluminum, and ferric chlorides. In summarizing their work, they point out: (1) the rate at which aged basic aluminum chloride solutions react with hydrochloric acid was found to decrease with increasing age of the solution; (2) the velocity of olation increases on increasing the basicity of aluminum chloride solution from 20 to 33.33 per cent, other factors remaining constant; and (3) the velocity of olation and free-acid formation is less with basic aluminum chloride solutions than with corresponding basic chromic chloride solutions.

The Aluminum Sulfate System

Since the various alums or aluminum sulfates are often used in the leather and fur manufacturing industries, a survey of the existing literature is of interest. In 1936, Theis and Schafer[10] began an investigation of aluminum sulfate

pickles. They first investigated the aluminum sulfate system in the absence of any type of salt. Their experimental procedure was as follows.

Animal skin properly beamed and bated was cut into small cubes and surface-dried. Exactly 50 grams of these cubes were placed in 250 ml of the various aluminum sulfate solutions. The solutions contained $Al_2(SO_4)_3 \cdot 18H_2O$ and the concentration varied from 1.0 grams to 100 grams per liter. The cubes were pickled for 24 hours, with constant agitation, at room temperature; they were then removed from the pickle liquor, drained, surface-dried and weighed. The residual liquor was analyzed for Al_2O_3, SO_3, basicity, and pH value. The data obtained are shown in Tables 280, 281, and 282 and in Figure 188.

Table 280

$Al_2(SO_4)_3 \cdot 18H_2O$ g/l.	$Al_2(SO_4)_3 \cdot 18H_2O$ g/250 cc.	% gain (swelling)	G total original Al_2O_3	G total residual Al_2O_3	G total original SO_3	G total residual SO_3
1	0.25	−12.0	0.038	0.019	0.090	0.054
5	1.25	− 2.8	.191	.055	.451	.156
10	2.50	− 1.0	.382	.107	.902	.419
15	3.75	+ 1.2	.574	.194	1.352	.504
20	5.00	+ 2.2	.766	.398	1.800	.973
25	6.25	+ 5.0	.956	.495	2.252	1.136
30	7.50	+ 7.6	1.147	.646	2.703	1.380
40	10.00	+ 8.8	1.530	.954	3.602	1.995
50	12.50	+11.2	1.912	1.311	4.509	2.619
60	15.00	+15.0	2.296	1.561	5.402	3.011
70	17.50	+17.5	2 680	1.999	6 308	3.930
80	20.00	+15.6	3.060	2.328	7.220	4.461
90	22.50	+16.8	3.445	2.676	8.110	5.131
100	25.00	+16.0	3.822	3.014	9.020	5.188

Table 281

$Al_2(SO_4)_3 \cdot 18H_2O$ g/l	% Initial Basicity	% Final Basicity	Initial pH	Final pH	Ratio of mols OH to mol Al_2O_3 from initial basicity
1	−22.6	−16.11	3.55	6.91	....
5	+ 0.52	−14 26	3.47	5.65	0.032
10	+ 0 52	−13.90	3.38	4.22	.031
15	+ 0.53	−11.65	3.28	3.89	.032
20	+ 0.51	− 6.67	3.19	3.86	.030
25	+ 0 55	− 1.16	3.15	3.80	.029
30	+ 1.63	+ 4.83	3.12	3.73	.095
40	+ 3.76	+ 5.50	3.09	3.66	.250
50	+ 6.48	+ 6.83	3.07	3.54	.389
60	+ 9.50	+ 8.34	3.06	3.51	.571
70	+10.92	+ 9.49	3.04	3.48	.606
80	+11.91	+10.37	3.03	3.42	.710
90	+12.52	+10.70	3.01	3.39	.750
100	+13.30	+11.80	3.00	3.35	.798

Table 282

g/l	Experimental $\frac{\text{Mol } SO_3}{\text{Mol } Al_2O_3}$	Calculated $\frac{\text{Mol } (3+x \text{ or } SO_3)}{Al_2O_3}$	x	y	Comments on spent liquor
1	3.71	3+.48		0.71	(very heavy white ppt.)
5	3.60	3+.43		.60	(heavy ppt.)
10	3.49	3+.42		.49	(medium ppt.)
15	3.31	3+.35		.31	(slight ppt.)
20	3.11	3+.20		.11	
25	2.92	2.93	0.080		
30	2.72	2.71	.280		
40	2.665	2.66	.325		
50	2.549	2.59	.451		
60	2.460	2.50	.540		
70	2.501	2.43	.499		
80	2.450	2.38	.550		
90	2 452	2.36	.548		
100	2.442	2.29	.558		

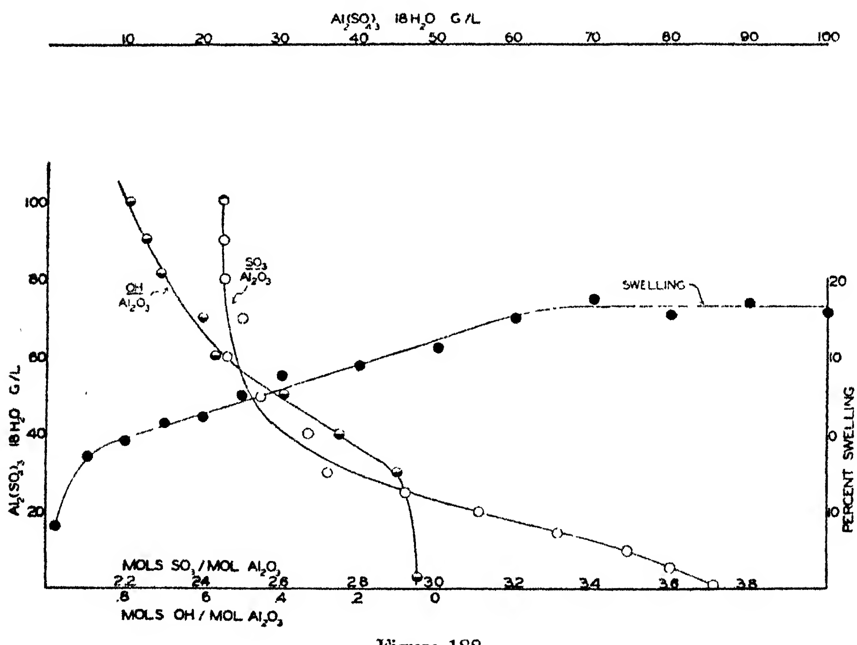


Figure 188

The analysis of the aluminum sulfate used in this study indicated a formula of $Al_2(OH)_{.04}(SO_4)_{2.96}$. It can readily be seen that at the lower concentrations more Al_2O_3 than SO_3 is taken up by the skin, whereas at high concentrations, this condition is reversed. At a concentration approximating 23.0 grams of $Al_2(SO_4)_3 \cdot 18H_2O$ per liter, equal amounts of Al_2O_3 and SO_3 are adsorbed.

When more SO_3 is taken up by the skin, the chemical constitution of the residual liquor with respect to the Al_2O_3 and SO_3 content assumes the relation $Al_2(OH)_x(SO_3)_{3-x}$, whereas for the case of greater adsorption of Al_2O_3, this

Table 283 Series 1 (Initially 0.3822 g Al_2O_3 and 0.9020 g SO_3 due to $Al_2(SO_4)_3 \cdot 18H_2O$)
10 grams $Al_2(SO_4)_3 \cdot 18H_2O$/liter

g/l Na_2SO_4	0	10	20	30	40	50	60	70	80	90	100
Initial pH	3.37	3.41	3.49	3.54	3.57	3.61	3.64	3.67	3.69	3.70	3.71
Final pH	3.95	4.16	4.25	4.26	4.27	4.27	4.28	4.29	4.35	4.37	4.38
% Gain in wt.	−2.6	−2.2	−2.2	−2.8	−5.0	−7.0	−9.6	−10.6	−11.8	−12.8	−14.2
Final total Al_2O_3	0.117	0.128	0.137	0.168	0.187	0.195	0.203	0.211	0.226	0.246	0.271
Final total SO_3	0.354	1.432	2.200	4.170	5.470	6.710	7.928	8.995	10.85	11.40	11.70
% Final Basicity	−9.65	−8.24	−3.49	−2.34	+1.37	+2.01	+4.02	+8.00	+8.23	+9.20	+12.15
Calc. mol ratio SO_3/Al_2O_3	3.58	3.49	3.21	3.14	2.92	2.88	2.75	2.52	2.51	2.45	2.27
G Na_2SO_4 absorbed	. . .	0.59	1.71	1.03	1.05	1.37	1.71	2.26	2.83	3.13	4.92

Table 284. Series 2 (Initially 0.8760 g Al_2O_3 and 2.065 g SO_3 due to $Al_2(SO_4)_3 \cdot 18H_2O$)
22.99 grams $Al_2(SO_4)_3 \cdot 18H_2O$/liter

g/l Na_2SO_4	0	10	20	30	40	50	60	70	80	90	100
Initial pH	3.23	3.33	3.44	3.50	3.53	3.56	3.57	3.59	3.60	3.61	3.62
Final pH	3.77	3.83	3.89	3.91	3.59	3.96	3.97	3.98	3.97	3.98	3.98
% Gain in wt.	+1.2	−0.6	−2.2	−4.0	−6.2	−7.0	−8.6	−9.8	−10.8	−12.6	−12.4
Final total Al_2O_3	0.553	0.565	0.585	0.596	0.611	0.625	0.638	0.654	0.666	0.694	0.705
Final total SO_3	1.44	2.48	3.68	4.78	6.21	7.55	9.00	10.09	11.39	12.73	13.96
% Final Basicity	+1.89	−1.86	−2.13	−3.34	−5.96	−6.31	−8.39	−9.17	−9.87	−10.21	−10.83
Calc. mol ratio SO_3/Al_2O_3	3.89	3.11	3.13	3.20	3.36	3.38	3.50	3.55	3.59	3.61	3.63
G Na_2SO_4 absorbed	. . . .	0.53	0.99	1.69	1.83	2.04	2.13	2.81	3.13	3.41	3.82

Table 285. Series 3 (Initially 1.9120 g Al_2O_3 and 4.5090 g SO_3 due to $Al_2(SO_4)_3 \cdot 18H_2O$)
50 grams $Al_2(SO_4)_3 \cdot 18H_2O$/liter

G/l Na_2SO_4	0	10	20	30	40	50	60	70	80	90	100
Initial pH	3.18	3.25	3.25	3.29	3.32	3.34	3.36	3.37	3.37	3.38	3.39
Final pH	3.49	3.59	3.61	3.63	3.66	3.68	3.69	3.70	3.70	3.71	3.73
% Gain in wt.	+6.0	+5.8	−0.2	−0.6	−2.0	−1.8	−3.0	−3.8	−5.8	−5.8	−6.6
Final total Al_2O_3	1.44	1.46	1.48	1.49	1.52	1.55	1.55	1.57	1.58	1.60	1.59
Final total SO_3	2.84	3.75	5.36	6.84	7.99	9.36	10.51	11.50	13.24	14.30	15.79
% Final Basicity	+10.5	+10.0	+5.3	+5.1	+5.2	+4.2	+3.8	+3.5	−3.7	−5.4	−10.3
Calc. mol ratio SO_3/Al_2O_3	2.27	2.30	2.68	2.69	2.69	2.75	2.77	2.79	3.22	3.33	3.62
G Na_2SO_4 absorbed	....	0.50	1.00	.94	1.49	1.79	2.31	3.18	3.59	4.55	5.00

Table 286. Series 1 (Initially 0.3822 g Al_2O_3 and 0.9020 g SO_3 total)
10 grams $Al_2(SO_4)_3 \cdot 18H_2O$/liter

G/l NaCl	0	10	20	30	40	50	60	70	80	90	100
Initial pH	3.41	3.47	3.50	3.51	3.51	3.49	3.49	3.48	3.46	3.44	3.42
Final pH	3.96	3.75	3.97	4.00	4.02	4.04	4.05	4.03	3.98	3.96	3.93
% Gain	−2.0	−5.0	−7.8	−9.4	−10.4	−10.8	−10.2	−11.8	−11.0	−12.0	−12.0
Final Al_2O_3	0.125	0.131	0.160	0.179	0.173	0.134	0.169	0.178	0.179	0.182	0.188
Final SO_3	0.324	0.348	0.424	0.500	0.530	0.492	0.456	0.484	0.477	0.457	0.455
Exp. ratio SO_3/Al_2O_3	3.31	3.40	3.38	3.57	3.92	4.66	3.45	3.48	3.39	3.21	3.09
% Basicity	−8.95	−9.66	−11.0	−12.2	−15.8	−19.4	−12.7	−9.98	−9.00	−9.48	−6.83
Calc. ratio	3.67	3.58	3.66	3.73	3.95	4.16	3.76	3.60	3.54	3.57	3.41
Final total NaCl	0.08	1.58	4.82	5.95	7.95	9.96	12.0	14.1	16.4	18.2	20.2
G NaCl absorbed	−0.08	0.92	0.17	1.55	2.05	2.54	3.00	3.44	3.62	4.30	4.80

Table 287. Series 2 (Initially 0.8760 g Al_2O_3 and 2.065 g SO_3 total)
22.9 grams $Al_2(SO_4)_3 \cdot 18H_2O$/liter

G/1 NaCl	0	10	20	30	40	50	60	70	80	90	100
Initial pH	3.24	3.24	3.24	3.22	3.20	3.18	3.15	3.12	3.10	3.09	3.05
Final pH	3.82	3.80	3.77	3.76	3.75	3.74	3.71	3.71	3.70	3.66	3.62
% Gain	+2.2	+1.0	+1.0	−1.4	−5.0	−7.2	−8.2	−11.4	−12.2	−14.0	−14.2
Final Al_2O_3	0.539	0.580	0.575	0.577	0.598	0.584	0.574	0.583	0.559	0.545	0.541
Final SO_3	1.25	1.42	1.46	1.44	1.51	1.50	1.54	1.53	1.49	1.46	1.49
Exp. ratio SO_3/Al_2O_3	2.95	3.11	3.23	3.19	3.22	3.28	3.42	3.35	3.40	3.41	3.50
% Basicity	−1.84	+1.50	+2.13	+1.34	+1.67	−1.50	−3.16	−4.33	−5.00	−6.07	−5.85
Calc. ratio	3.11	2.91	2.87	2.92	2.90	3.09	3.19	3.26	3.30	3.37	3.35
Final total NaCl	0.20	2.02	3.95	5.26	7.95	9.72	11.78	13.92	16.14	18.20	20.30
G NaCl absorbed	−0.20	0.42	1.06	2.24	2.05	2.78	3.22	3.58	3.86	4.30	4.70

Table 288. Series 3 (Initially 1.912 g Al_2O_3 and 4.5090 g SO_3 total)
50 grams $Al_2(SO_4)_3 \cdot 18H_2O$/liter

G/1 NaCl	0	10	20	30	40	50	60	70	80	90	100
Initial pH	3.18	3.17	3.15	3.14	3.11	3.11	3.10	3.09	3.06	3.01	2.97
Final pH	3.52	3.50	3.50	3.48	3.45	3.45	3.40	3.39	3.39	3.37	3.34
% Gain	+6.8	+4.8	+2.2	+0.2	−2.0	−4.4	−6.2	−6.2	−9.4	−9.0	−10.2
Final Al_2O_3	1.45	1.45	1.51	1.50	1.49	1.58	1.51	1.59	1.58	1.56	1.59
Final SO_3	2.75	2.62	2.58	2.63	2.46	2.83	3.01	3.17	3.39	3.56	3.28
Exp. ratio SO_3/Al_2O_3	2.40	2.31	2.19	2.25	2.10	2.36	2.54	2.71	2.73	2.90	2.64
% Basicity	+6.8	+5.8	+9.7	+11.3	+13.3	+15.8	+14.2	+13.9	+9.8	+3.5	+8.0
Calc. ratio	2.59	2.65	2.42	2.32	2.20	2.05	2.15	2.11	2.41	2.79	2.52
Final total NaCl	0.31	1.98	3.97	6.24	7.9	9.9	12.0	14.3	16.4	18.3	20.6
G NaCl absorbed	−0.31	0.52	1.03	1.26	2.12	2.65	3.00	3.20	3.62	4.18	4.40

relation becomes $Al_2(SO_4)_3 + yH_2SO_4$. The data show the molar ratio of OH/Al_2O_3 and SO_3/Al_2O_3 together with the swelling. At low concentrations, negative swelling results, but at concentrations greater than 12.0 grams of $Al_2(SO_4)_3 \cdot 18H_2O$ per liter positive swelling is found.

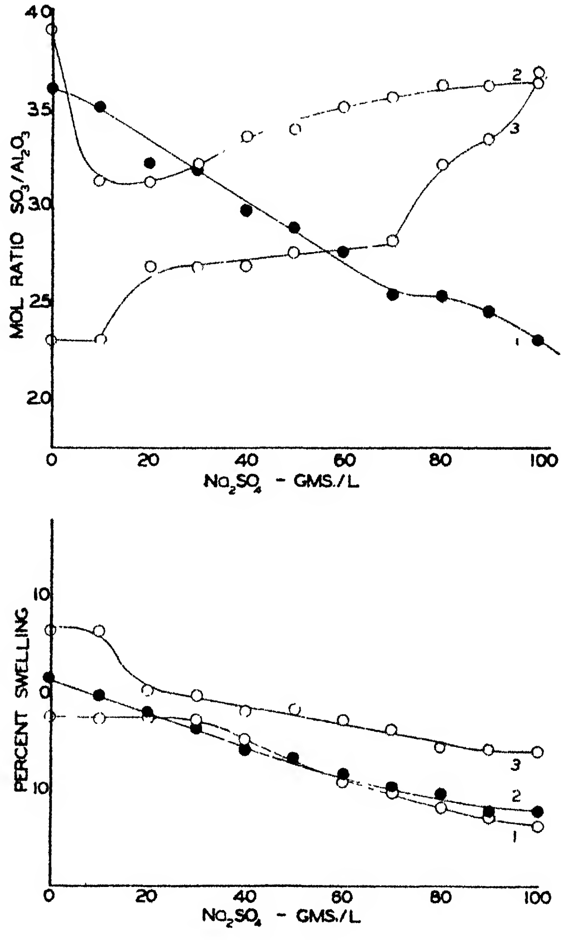


Figure 189

In a later study, Theis and Schaffer investigated the $Al_2(SO_4)_3$-Na_2SO_4 and the $Al_2(SO_4)_3$-NaCl systems. Their experimental procedure was the same as that already described. These concentrations of aluminum sulfate [$Al_2(SO_4)_3 \cdot 18H_2O$] were used, namely, 10, 22.9, and 50 grams per liter. The salt was varied between 0 and 100 grams per liter. Tables 283 to 288 and Figure 189 show the data obtained.

These data indicate a real difference between the pure aluminum sulfate pickle and the aluminum sulfate-neutral salt pickles. When aluminum sulfate was used alone, more SO_3 than Al_2O_3 was adsorbed as the concentration of the aluminum sulfate increased. The data show that addition of neutral salt to the aluminum sulfate solution causes a greater adsorption of Al_2O_3 than of SO_3. Figure 189 gives the data in graphical form and indicates the following trends: curve 1, representing 10 grams per liter of $Al_2(SO_4)_3 \cdot 18H_2O$, shows that there is greater adsorption of Al_2O_3 up to 40 grams per liter of Na_2SO_4; curve 2, representing 22.9 grams of $Al_2(SO_4)_3 \cdot 18H_2O$ per liter, indicates greater adsorption of Al_2O_3 by the skin at Na_2SO_4 concentrations greater than 10 grams per liter; and curve 3 (50 grams per liter of $Al_2(SO_4)_3 \cdot 18H_2O$) shows greater adsorption of SO_3 than Al_2O_3 by the skin at Na_2SO_4 concentrations less than 75 grams per liter.

When sodium chloride was used in place of sodium sulfate, the same trends are found, namely, that as the sodium chloride is increased more Al_2O_3 is adsorbed by the skin.

If we compare the alum-salt pickling data with those obtained by the acid-salt process, we find a distinct difference. In the acid-salt systems, increasing salt content not only repressed swelling but also caused increased acid adsorption. In the alum-salt systems, a different picture is presented. The data of Theis and Schafer definitely show that as neutral salt concentration increases, more Al_2O_3 is taken up by the skin than acid. Thus, in contradistinction to the acid-salt systems, in which acid adsorption is increased by added salt, in the alum-salt systems, added neutral salt causes preferential adsorption of the Al_2O_3, leaving the residual pickle liquor more acid in character. Since neutral salts cause such preferential adsorption of the Al_2O_3 (or basic aluminum salt) rather than acid, it may very well be one of the reasons why the addition of neutral salts causes increased tanning action of the basic aluminum sulfate pickles

Organic Acid-Aluminum Complexes

In the case of iron and chromium tannages, organic acids or their salts have been shown to be of real and significant value. Numerous investigators have indicated the value of adding citrates, acetates, etc., to aluminum sulfate systems. In 1935, Wilson, Peng, and Li[13] studied the tanning action of the aluminates. They first studied sodium dioxalato-diaquo-aluminate. They prepared this compound by precipitating aluminum hydroxide with ammonia from aluminum sulfate. Normal aluminum oxalate was then prepared by digesting the freshly precipitated aluminum hydroxide with a solution of oxalic acid. Sodium oxalate was then added in proper quantity to produce the dioxalato- compound. The probable reaction is as follows:

$$Al_2(C_2O_4)_3 + Na_2C_2O_4 \longrightarrow 2NaAl(C_2O_4)_2$$

Stock solutions of the dioxalato- compound were prepared and standard hide powder was tanned in diluted portions of this solution. Their results are shown in the following tables and figures. (Text continued on p. 676)

Table 289. Effect of Concentration.

Gm Al_2O_3 per liter	pH value Before	pH value After	Mg Al_2O_3 fixed by 2 gm Hide Powder	Gm Al_2O_3 per 100 gm Collagen	Character of Tanned Powder
0.5	4.63	4.59	7.0	0.413	Fair
1.0	4.63	4.52	22.2	1.309	Fair
2.0	4.63	4.45	37.0	2.183	Good
2.5	4.63	4.48	53.6	3.162	Good
5.0	4.63	4.46	79.1	4.672	Good
7.5	4.63	4.46	69.3	3.890	Good
10.0	4.63	4.49	54.4	3.240	Good
12.5	4.63	4.42	51.6	3.040	Good
30.0	4.63	4.32	48.7	2.870	Good

Table 290. Effect of pH Value.

(Concentration = 5 gm Al_2O_3/liter. Time = 48 hours)

pH Value Initial	pH Value Filtrate	Mg Al_2O_3 Fixed per 2 gm Hide Powder	Gm Al_2O_3 per 100 gm Collagen	Character of Tanned Powder
3.0	3.55	19.6	1.154	Poor
4.0	4.05	25.8	1.540	Fair
4.5	4.50	44.8	2.640	Good
5.0	4.91	180.8	10.640	Very good
6.0	5.95	135.4	7.980	Very good
7.0	6.86	49.4	2.910	Fair
8.0	7.75	9.8	0.577	Poor

Table 291. Effect of NaCl.

(pH = 5 Concentration = 5 gm Al_2O_3/liter. Time = 48 hours)

NaCl (mole/liter)	pH of Filtrate	Gm Al_2O_3 per 100 gm Collagen	Character of Tanned Powder
0.0	4.99	5.26	Very good
0.2	4.77	4.74	Very good
0.4	4.8	4.12	Good
0.6	4.68	4.03	Good
0.8	4.77	2.98	Good
1.0	4.76	2.65	Good
2.0	4.71	3.01	Good
3.0	4.69	2.86	Good

Table 292. Tanning Sheep Skin; Comparison of Various Methods Used for Tanning

	Both skins and solution at pH 5.0: Fat-liquored	Both skins and solution at pH 5.0: No Fat-liquoring	Pickled Tanned without pH adjustment	Pickled Tanned at pH 5.0	Aluminate Retanned Pure Alum Leather
Description of leather	Horny on drying soft on staking stretching medium pure white	Horny on drying raggy on staking stretching good white	Resemble raw skin	Condition is worse than no pickling	Same as an alum tanned leather
Shrinkage temperature (° C)		72	55	60	75

Table 293. Effect of Added Sodium Tartrate on Basic Aluminum Sulfate Solution.*

Exp. No	$Al(OH)SO_4$ Soln. (cc)	Mole Ratio Salt to Al	Gms Salt	Migration	pH	Ppt. Fig.
1	400	0.00	0.00	Cathodic	3.67	11.67
2	400	0.25	11.52	Anodic	3.73	22.50
3	400	0.50	23.04	Anodic	3.59	26.43
4	400	1.00	46.08	Anodic	3.31	
5	400	1.50	69.12	Anodic	3.38	
6	400	3.00	138.24	......	4.35	....

* The concentration of aluminum solutions used here was approximately 0.5N with respect to Al.

Table 294. Tanning Tests.

Exp. No.	Appearance of Tanned Hide Powder	Appearance of Solution — Immediately	After 1 Week	After 1 Month
1	Gelatinized	Clear	Clear	Clear
2	(Very fluffy) (Well tanned)	Clear	Clear	Clear
3	(Very fluffy) (Well tanned)	Clear	Clear	Clear
4	(Very fluffy) (Well tanned)	Clear	Clear	Clear
5	Fluffy	Clear	Clear	Clear
6	Soft	Ppt. of $Al(OH)_3$ with crystals of salt	Same as before	Same as before

Table 295. Effect of Added Sodium Acetate on Basic Aluminum Sulfate Solution.

Exp No	$Al(OH)SO_4$ Soln (cc)	Mole Ratio Salt to Al	Gms. salt	Migration	pH	Ppt. Fig.
1	400	0.00	0.00	Cathodic	3.67	11.67
2	400	0.25	3.45	Anodic	3.84	11.71
3	400	0.50	6.90	Anodic	3.87	11.07
4	400	1 00	13.80	Anodic	3.94	11.02
5	400	1 50	17.25	Anodic	3.23	10.61
6	400	3.00	34.50	Anodic	3.48	11.02

Table 296. Tanning Tests.

Exp No	Appearance of Tanned Hide Powder	Appearance of Solution — Immediately	After 1 Week	After 1 Month
1	Gelatinized	Clear	Clear	Clear
2	Fluffy	Clear	Clear	Clear
3	Fluffy	Clear	Clear	Clear
4	Fluffy	Clear	Clear	Ppt.
5	Soft	Clear	Clear	Heavy ppt.
6	Soft	Clear	Turbid	Heavy ppt.

Table 297. Effect of Added Sodium Formate on Basic Aluminum Sulfate Solution.

Exp No.	$Al(OH)SO_4$ Soln. (cc)	Mole Ratio Salt to Al	Gms Salt	Migration	pH	Ppt. Fig.
1	400	0.00	0.00	Cathodic	3.67	11.67
2	400	0.25	6.81	Anodic	3.87	14.74
3	400	0.50	13.62	Anodic	3.92	12.58
4	400	1.00	27.24	Anodic	3.67	14.70
5	400	1.50	34.05	Anodic	4.20	12.14
6	400	3.00	68.10	Anodic	4.82	12.10

Table 298. Tanning Tests.

Exp. No.	Appearance of Tanned Hide Powder	Appearance of Solution Immediately	After 1 Week	After 1 Month
1	Gelatinized	Clear	Clear	Clear
2	Fluffy	Clear	Clear	Clear
3	Fluffy	Clear	Clear	Clear
4	Soft	Clear	Turbid	Heavy ppt.
5	Soft	Clear	Heavy ppt.	Heavy ppt.
6	Horny	Clear	Heavy ppt.	Heavy ppt.

Table 299. Effect of Concentration.

Total Vol (cc)	Conc. (gm Al_2O_3/liter)	pH Initial	pH Final	Gm Al_2O_3 fixed per 10 gm col	Character of tanning
150	0.50	5.00	5.00	0.485	Fair
150	1.00	5.00	4.98	0.520	Fair
150	2.50	5.00	4.89	0.616	Good
150	3.00	5.00	5.00	0.734	Good
150	5.00	5.00	5.00	0.948	Good
150	7.00	5.00	4.97	0.757	Good
150	10.00	5.00	5.00	0.704	Fair
150	20.00	5.00	4.95	0.722	Fair

Table 300. Effect of pH Value.

Total Vol. (cc)	Conc. (gm Al_2O_3/liter)	pH Initial	pH Final	Gm Al_2O_3 fixed per 100 gm col	Character of tanning
150	5.00	3.00	2 82	0.857	Horny
150	5.00	3.50	3.00	1.182	Fair
150	5.00	3.70	3.68	1.405	Good
150	5.00	4.00	3.85	2.680	Good
150	5.00	4.50	4.40	1 424	Good
150	5.00	5 00	4.98	0.668	Fair
150	5.00	6.00	5.55	0 745	Fair
150	5.00	7.00	7.55	0.616	Hard
150	5 00	8.00	8.03	0.704	Horny
150	5.00	9.00	8.90	0.704	Horny

Table 301. Tanning Sheep Skin with Basic Aluminum Sulfate together with Different Mole Ratios of Sodium Tartrate and Sodium Acetate.

$Al(OH)SO_4$ Soln (cc)	Moles Tartrate and Acetate to Al	Gms Tartrate	Gms Acetate
200	0.25	5.76	3.40
200	0.50	11.52	6 81
200	1.00	23.04	13.62

Shrinkage Temperature (° C)	Character of Tanning
81.0	Horny on drying; soft on staking; stretching medium; pure white.
82 0	Horny on drying; soft on staking; stretching good; pure white.
85.0	Horny on drying; soft on staking; stretching good; pure white.

Table 302. Tanning Sheep Skin with Basic Aluminum Sulfate together with Different Mole Ratios of Sodium Tartrate and Sodium Formate.

$Al(OH)SO_4$ Soln. (cc)	Moles Tartrate and Formate to Al	Gms Tartrate	Gms Formate
200	0.25	5.76	1.73
200	0.50	11.52	3.45
200	1.00	23.04	6.90

Shrinkage Temperature (° C)	Character of Tanning
82.0	Horny on drying; raggy on staking; stretching medium; pure white.
84.0	Horny on drying; soft on staking; stretching medium; pure white.
84 0	Horny on drying; soft on staking; stretching medium; pure white.

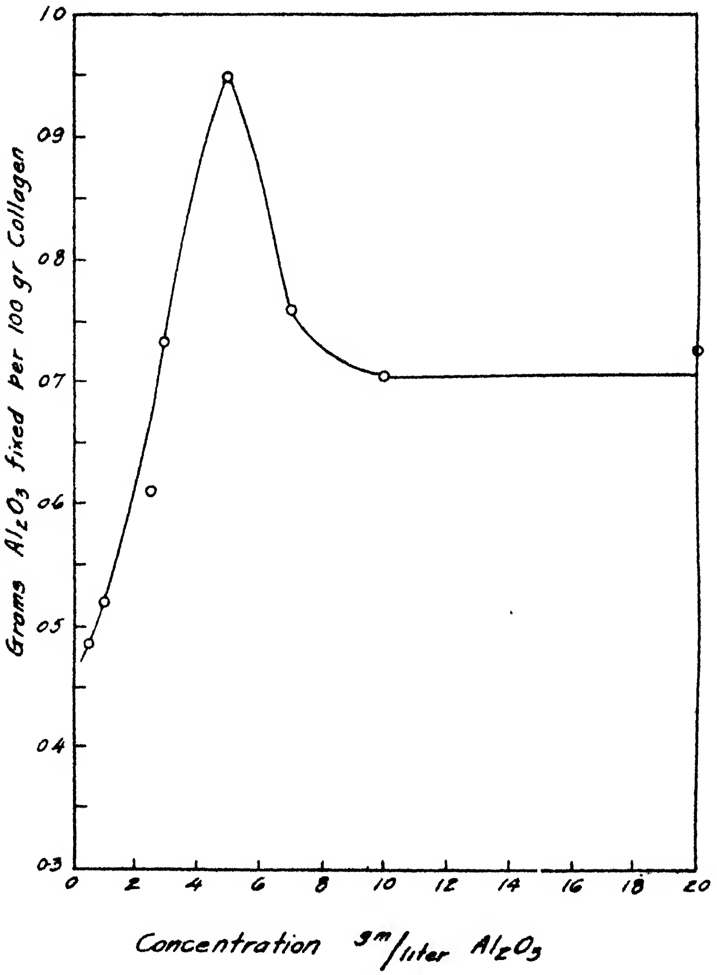


Figure 190. Effect of Concentration, Oxalato Aluminate

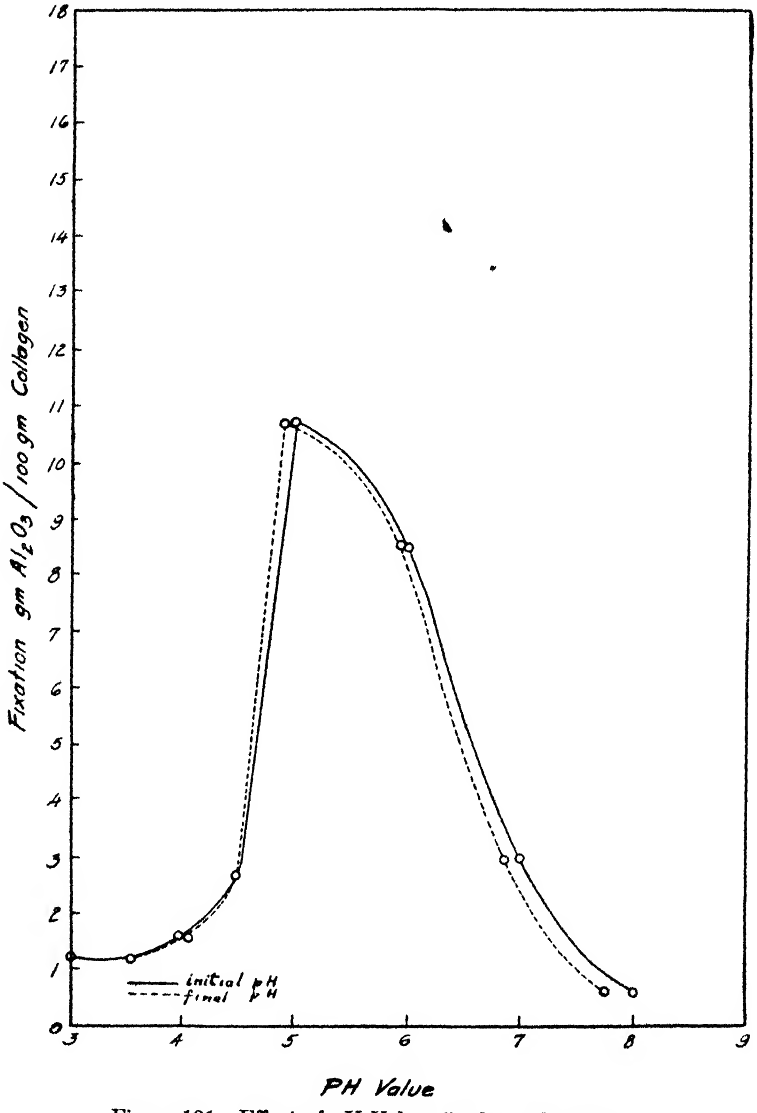


Figure 191 Effect of pH Value, Oxalato Aluminate

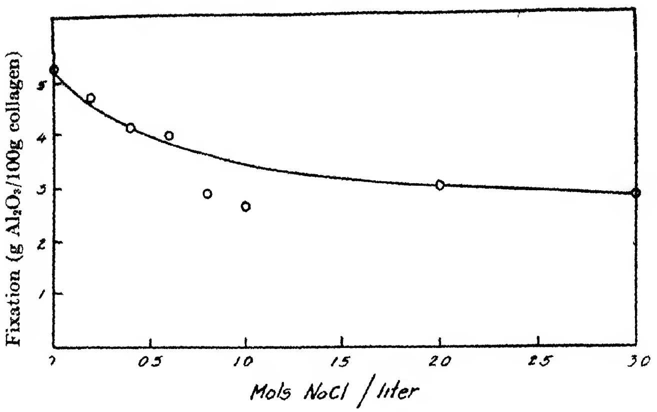


Figure 192 Effect of Added Salt, Oxalato Aluminate

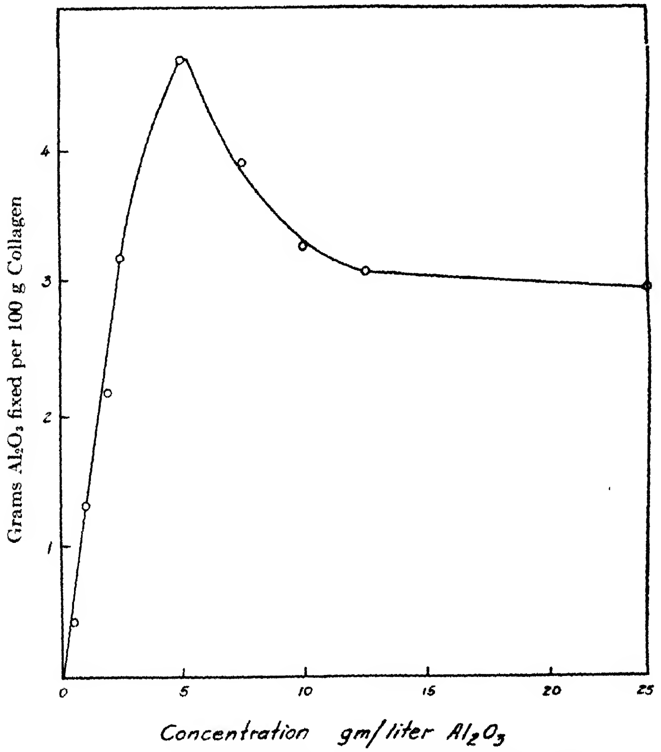


Figure 193 Effect of Concentration Tartarato Aluminate

Wilson, Peng and Li investigated the effect of masking agents such as sodium tartrate, acetate and formate on basic aluminum sulfate. They prepared a 33.33 per cent basic aluminum sulfate solution by treating normal aluminum sulfate with sodium hydroxide. The alkali was added dropwise

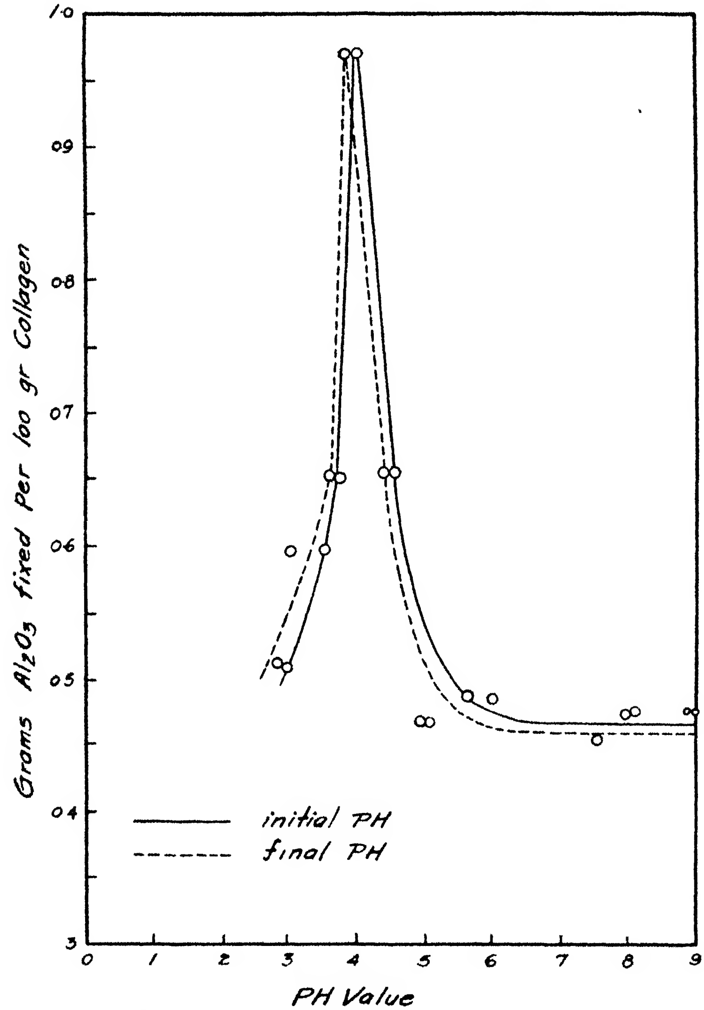


Figure 194 Effect of pH Value Tartarato Aluminate

and with constant stirring. Two hundred-ml portions of the basic aluminum sulfate stock solution were placed in flasks and the calculated quantities of sodium tartrate, sodium acetate and sodium formate were added. The precipitation figure and direction of migration of the resulting solutions were

determined. Two grams of hide powder were placed in a series of flasks, each containing 200 ml of the above solutions. After several hours' agitation, the hide powder was removed from the solution and visually examined for tannage.

These same investigators studied the tanning action of sodium ditartarato-diaquo-aluminate. They prepared this anionic aluminum compound in the same way as described for the dioxalato- compound previously described. The effects of concentration and pH value are shown in the tables and figures.

Wilson, Peng, and Li[13] point out that the maximum fixation of aluminum in the region of the isoelectric point confirms Gustavson's opinion, namely, that the fixation of anionic metallic complexes is due to the secondary valencies

Table 303. Fifty cc of Aluminum Sulfate Solution Treated With 1.0*N* Salt Solution. (Values given are expressed in pH)

Vol added (cc)	K_2SO_4	KCl	NaCl	Pure H_2O
0	3.39	3.39	3 39	3.39
5	3.54	3.49	3 43	3.40
10	3.67	3.51	3.45	3.42
15	3 75	3.52	3 47	3.45
20	3.81	3.54	3.49	3.47
25	3.86	3.57	3.50	3.50
30	3.91	3.59	3 52	3.52
35	3.96	3.61	3.54	3.54
40	4.00	3 63	3.56	3.57
45	4 03	3.64	3.57	3.59
50	4 05	3.66	3 59	3.61

Table 304. Fifty cc of Aluminum Chloride Solution Treated With 1.0*N* Salt Solution. (Values given are expressed in pH)

Vol added (cc)	K_2SO_4	KCl
0	4.14	4.14
5	4.18	4.16
10	4.25	4.17
15	4.30	4.20
20	4.35	4.22
25	4.41	4.24
30	4.43	4.27
35	4.44	4.28
40	4.47	4 30
45	4.51	4.32
50	4 53	4.34

Table 305. Fifty cc of Aluminum Sulfate Solution Treated with Solid Salt (Values given are expressed in pH)

Equivalents salt added	NaCl	Anhyd Na_2SO_4
0.0	3 39	3.39
0.5	3.31	3.66
1.0	3.20	3.73
1.5	3.11	3.81
2 0	3.03	3.86
2.5	2.99	...
3.0	2.81	...

of the protein, which are at a maximum in the isoelectric zone. Their data show a maximum fixation at pH 5.0 for the oxalato- compounds and a maximum binding at pH 4.0 for the tartarato- complexes. The rise to the maximum values is very sharp and might point to a shift in the isoelectric point due to specific effects of the different salts. Anionic tannage with dioxalatochromiate gives a maximum Cr_2O_3 fixation at pH 5.0.

These investigators studied in a practical way the tannage of sheep skin by sodium ditartarato-aluminate alone and in the presence of such salts as sodium acetate and formate. They found that the ditartarato salt gave no tannage whatsoever. They found, however, that in the presence of acetate or formate, a satisfactory tannage resulted. They point out that since the mixtures are good tanning agents, penetration compounds of high molecular weight must be formed. A resume of their practical tests is shown in Tables 301 and 302.

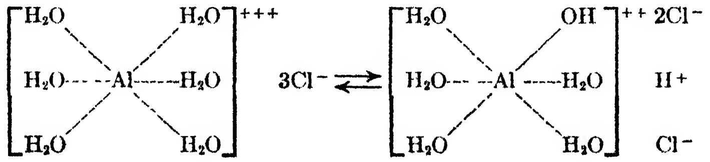
Effect of Neutral Salts on Solutions of Aluminum Salts

In the study of the various alum-salt systems, it was shown that the added neutral salt played an important role in the alum tannage. It was further postulated, that, since the added neutral salt caused increased Al_2O_3 adsorption rather than increased acid adsorption, the neutral salt effect was very important.

Thomas and Whitehead[12] made a study of the effect of neutral salts on the pH value of aluminum salt solutions. Their experimental method was to measure the pH values of aluminum sulfate and aluminum chloride solutions before and after the addition of various amounts of KCl, NaCl, K_2SO_4 and Na_2SO_4. For this experiment they used a 0.5 per cent Al_2O_3 solution. Their results are shown in Tables 303, 304 and 305 and in Figure 195.

Thomas and Whitehead[12] show (curves 1 and 2) that the hydrogen ion activity of the aluminum chloride solution is decreased by both sulfate and chloride ion when present in low concentrations. The same is true of aluminum sulfate solutions. The data apparently indicate that the sulfate ion is more effective than the chloride ion, but both increase the pH value of the solution. Dilution with water also increases the pH value. Solid sodium chloride decreases the pH value, whereas sodium sulfate increases it.

Thomas and Whitehead believe two factors are involved. The first has to do with the effect of the chloride ion on the basic aluminum salt; this may be pictured as follows:



This concept shows the formation of the basic aluminum chloride, the result of a coordinated aquo- group releasing a hydrogen ion to the outer solution, leaving a hydroxo- group attached to the nucleus. The nucleus of the pentaquo-hydroxo-alumini-chloride has now two positive charges.

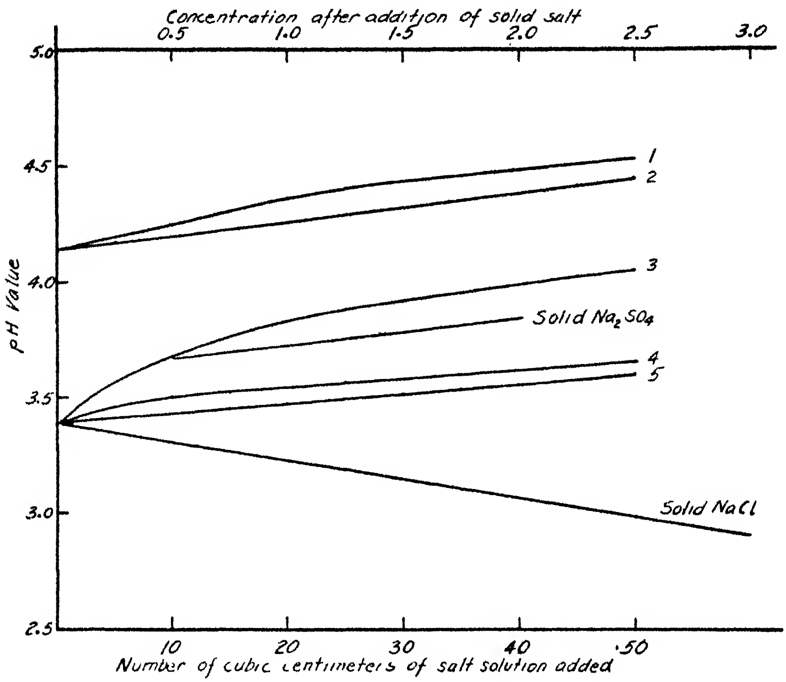


Figure 195

If chloride ions are now added to such a solution, they will tend to replace the hydroxo- groups coordinated with the aluminum complex; the hydroxo groups thus forced out from the complex will unite with H^+ ions in the outer solution to form water. This may be represented as follows:

$$\left[\begin{array}{ccc} H_2O & & OH \\ H_2O & Al & H_2O \\ H_2O & & H_2O \end{array}\right]^{++} \begin{array}{c} 2Cl^- \\ H^+ \\ Cl^- \end{array} + NaCl \rightleftarrows \left[\begin{array}{ccc} H_2O & & Cl \\ H_2O & Al & H_2O \\ H_2O & & H_2O \end{array}\right]^{++} \begin{array}{c} 2Cl^- \\ Na^+ \\ Cl^- \end{array} + H_2O$$

In this manner added sodium chloride will decrease the hydrogen ion activity and thus increase the pH value of the solution.

The second factor involved has to do with the removal of water molecules from the solvent by hydration of the Na^+ and Cl^- ions.

Thomas and Whitehead state that the presence of $SO_4^{=}$ ions more or less complicates the picture and that there are at least three factors to be considered. In the first place, there is the tendency of the $SO_4^{=}$ ions to replace hydroxo- groups of the Werner complex, and this in itself would increase the pH value of the solution. The sulfate ions would do this more effectively than chloride ions if the penetration order found by Stiasny and Balanyi[9] holds. This order has been found to be:

oxalate > acetate > formate > sulfate > chloride > nitrate

Another factor would be the probability of the sulfate ion uniting with the hydrogen ion to form hydrosulfate ion, $SO_4^{=} + H^+ \rightleftarrows HSO_4^-$, which would also tend to increase the pH value of the aluminum solutions.

As in the case of the chloride ion, there is the factor of hydration of the ions with a corresponding increase in H^+ activity. When $1.0N$ solutions were used, only the first two factors entered the picture, namely, the Werner substitution and the union of $SO_4^{=}$ ion with H^+ ion. When solid sodium sulfate was added, the factor of hydration did enter, and the total effect on the aluminum solution was the net effect of all three factors. Since the first two factors cause an increase in the pH value of the solution and the last a decrease in this value, the effect of the hydration of the Na^+ and $SO_4^{=}$ ions is less than that of the dilute solution in the case of sulfate.

Theory of Aluminum Tannage

Cameron and McLaughlin[1] have postulated that cationic chrome tanning is a reversible deposition process involving the taking up of acid by the skin protein and the deposition of a 66.66 per cent basic compound regardless of the basicity and pH value of the liquor. The existing data relating to aluminum tannage would seem to indicate that similar reasoning would apply. McLaughlin and Adams and Theis have shown, for chrome tanning, that acid-saturated skin proteins will not fix chromium under any known conditions. Similarly, acid-saturated skin will not fix aluminum, since it has been found in practice that Al_2O_3 fixation occurs best at about pH 5.0.

Wilson and Yu[14] have attempted to apply the reasoning of Cameron and McLaughlin to their studies of basic aluminum sulfate. Their experimental procedure was in the main similar to that used by Cameron and McLaughlin in their chrome-tanning studies and the reader is referred to Chapter 15 for specific details. Wilson and Yu prepared neutral salt-free basic aluminum sulfate by treating the normal salt with definite quantities of barium hydroxide. The reaction is probably:

$$2Al_2(SO_4)_3 + Ba(OH)_2 \longrightarrow 2AlOHSO_4 + BaSO_4$$

They prepared solutions of five different basicities, 0, 11.49, 22.5, 21.8 and 33.33 per cent.

Experimentally, these investigators used 7.0-gram samples of skin in 110 ml of solution for a 48-hour tanning period. Constant agitation at 25° was employed. After tanning, the skin was removed and pressed at 10,000 pounds per square inch. Moisture, aluminum, nitrogen, and sulfate were determined in the tanned skins and aluminum and sulfate in the equilibrium solution.

Wilson and Yu calculated their data similarly to Cameron and McLaughlin, and concluded that the method used by Cameron and McLaughlin to prove that the chrome compound fixed by hide substance is of 66.67 per cent basicity is of doubtful validity.

Cameron and McLaughlin[2] dispute the above statement regarding their studies, and point out that the doubts of Wilson and Yu are based upon errors of plotting and of calculation. Recalculating and replotting the data of Wilson and Yu shows beyond doubt that these errors exist. Cameron and McLaughlin further point out that due to the complicated structure of skin proteins, the closeness of their 69 points to a common line is noteworthy.

In Chapter 11, dealing with pickling, certain data were given relative to the alum-salt systems (Figures 101, 102, and 103). These data show the decided effect of the neutral salt, not only in the repression of the swelling but also upon the degree of leathering as indicated by the shrinkage temperature. They also show that alum-treated skin should be neutralized to a pH value greater than 4.5 in order that Al_2O_3 fixation can take place and thus give the necessary cohesive strength.

Alum tannage, at this time, must be considered in practically the same light as iron tannage, *i.e.*, much more study is necessary relative to the aluminum complex and its reaction with skin proteins. Undoubtedly, the organo-aluminum complexes play an entirely different role from the inorganic ones and a careful study of the anionic systems will yield interesting and profitable results.

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Chapter 21

Syntans

Since Kekulé proved that carbon is a tetravalent element and that in organic compounds the atoms are linked together in open-chain and also in closed-chain or ring form, tremendous strides have been made in the development of synthetic organic chemistry.

Natural products used for food, clothing, medicine and other necessities of life and civilization were investigated. Their chemical constitution was ascertained and methods were found to reproduce many of them synthetically.

Emil Fischer, Karl Freudenberg, and Max Bergmann were the great pioneers in the investigation of the tannins. They not only proved the chemical constitution of the principal components of some of the natural tanning agents, but also worked out methods for their synthetic reproduction. They showed that the active tanning components are very complicated poly-hydroxy benzoles or polymerides of same. Their colloidal solubility and their tanning action are due principally to the presence of a sufficient number of phenolic hydroxy groups. The solubility is improved and characteristic tanning actions are caused by the simultaneous presence of carboxyl groups. Consequently, if hide substance is exposed to a solution of vegetable-tanning agents, it is subjected to the influence of a mixture of molecular particles which are graduated from a highly dispersed to a coarsely dispersed state, and which are of a weakly acid nature. Since the absorption of the tanning agents takes place according to the countercurrent principle, the molecular particles are assumed to be discharged and absorbed, beginning with those of lowest dispersion and ending with those of highest.

Because of this, absorption of the tanning agent by the hide substance takes place progressively and uniformly; and if the right selection of tanning agents has been compounded, satisfactory results as to yield and physical properties are assured, if the tanning agent has been applied efficiently. But it is obvious that those tanning agents having, in proportion to the size of the molecule, the largest number of hydroxy and carboxyl groups and consequently being in a state of highest colloidal dispersion tan quickest, whereas those showing poor solubility and coarser dispersion tan more slowly but produce greater weight yield.

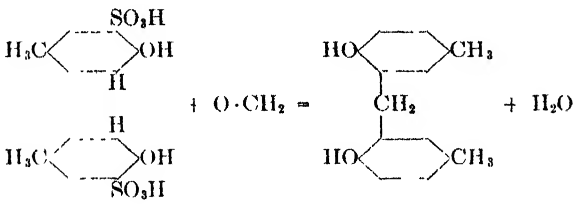
Although the chemical constitution of the principal active medium of some

of the natural tanning agents was known and was synthesized, it was obvious that a synthetic reproduction for practical purposes was out of the question on account of the prohibitive cost of the manufacturing process.

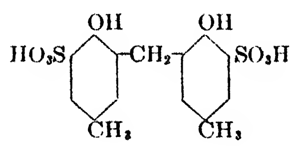
Edmund Stiasny patented September 7th, 1911 (Austrian Patent No. 58405): "A Method for the Synthetic Manufacture of Tanning Substances." The products manufactured by this process are not in any way related to natural tanning agents. However, they react similarly to the natural products, inasmuch as they precipitate gelatin, protect the hide substance which is treated with them against putrefaction, and produce a leather-like material of white color but of poor fastness to light. The hide substance so treated does not yield gelatin when heated with water.

Stiasny's patent claims comprise water-soluble formaldehyde condensation products of phenol or cresol sulfonic acids and also condensation products of phenol or cresol with subsequent sulfonation to obtain water solubility.

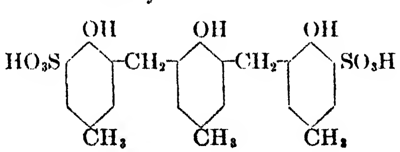
The following equation represents the course of the chemical reaction theoretically:



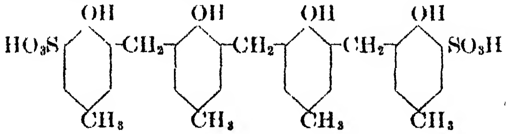
However, in a practical manufacturing way the course of the reaction is somewhat different. For example, commercial cresol is a mixture of the three isomers and consequently a mixture of ten isomer dihydroxy-ditolylmethan-disulfonic acids can be expected. Furthermore, when H. Schütte[6] investigated the formaldehyde condensation product of *p*-cresol-sulfonic acid, he found that during the condensation process at least three of the following complexes are formed:



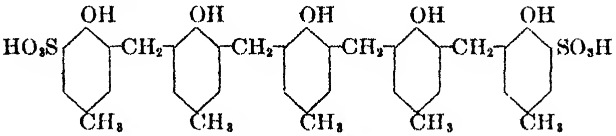
(1) *Dihydroxy-ditolyl-monomethan-disulfonic acid*



(2) *Trihydroxy-tritolyl-dimethan-disulfonic acid*



(3) *Tetrahydroxy-tetratolyl-trimethan-disulfonic acid*



(4) Pentahydroxy-pentatolyl-tetramethan-disulfonic acid

(1), (2), and (4) have been determined definitely by analysis.

We also find that with decreasing solubility these high molecular complexes become more sensitive to salt and to strong acids and show less dispersing properties than the simpler condensation products with a comparatively large percentage of sulfonic acid groups. The less water-soluble complexes, which are not sufficiently soluble by themselves, are kept in a state of colloidal dispersion by the more water-soluble ones.

All these phenomena demonstrate that in some respects a number of these condensation products with tanning properties approach conditions shown by solutions of vegetable-tanning agents.

Since the invention and practical application of these condensation products, great strides have been made in the field of artificial tanning agents.

Although the above mentioned comparatively simple complexes have definite tanning properties, the experience gained by practical application, scientific investigation and resulting deductions proved that they were suitable only as auxiliary tanning agents. Due to the comparatively small size of these molecules and their finely dispersed form, they are absorbed by the hide substance faster than are vegetable-tanning agents; and, as Carl Felzmann[1] showed, the absorption process is a true chemical reaction, *i.e.*, the sulfonic acid group interacts directly with the amino group of the hide substance.

The above formulas reveal that with the increase of the size of the molecule, the number of hydroxy groups increases while the number of sulfonic acid groups remains constant.

This chain-like formation of highly complex molecules indicates that with the decrease of the number of sulfonic acid groups in proportion to the increasing size of the molecules, the water-solubility of the compound decreases, notwithstanding the increase of the water-solubilizing hydroxy groups.

According to tests made by Wolesensky,[9] formaldehyde condensation products of cresol, sulfonated subsequently, yielded plumper leather than the products obtained from cresol sulfonic acid which is subsequently condensed with formaldehyde. This observation was quite correct. It is generally more difficult to obtain the same degree of water solubility by subsequent sulfonation. Consequently, in such a product a larger number of more or less insoluble particles are present in the form of colloidal dispersion than in a condensation product made from the respective sulfonic acids. Some of these dispersed particles may contain only one sulfonic acid group or none at all. In the latter case, and if they possess a sufficient number of hydroxy and/or other weakly acid groups, they would not interact with the amino groups of the hide substance but would react similarly to the phenolic compounds comprising the active medium of vegetable-tanning agents.

J. A. Wilson[8] expressed the opinion that syntans (auxiliary tanning agents), when used in combination with vegetable-tanning agents, diffuse into the skin more rapidly than the latter, attaching themselves to groups in the protein molecule that would otherwise fix tannins. The slower-moving tannins, not being fixed by groups already occupied by syntan, are then able to diffuse farther into the skin, thereby increasing the rate of diffusion of the tannins. The protein groups not occupied by syntan combine with the tannins all the more vigorously since the pH value of the tan liquor has been lowered by the syntan.

Schäfer[5] found that the tanning properties of tanning sulfonic acids (syntans) are improved as the acidity (sulfonic acid character) of the compound is decreased. In other words, the tanning properties improve with a decrease in the number of sulfonic acid groups in the molecule.

Küntzel and Schwank[2] thoroughly investigated the different reaction products obtained by gradual sulfonation of phenol and cresol-formaldehyde condensates (2 moles to 1 mole formaldehyde). They found that at least 50 per cent of anhydrous sulfuric acid is necessary to obtain unlimited water solubility. In other words, in a dihydroxy-diphenylmethane compound, at least one phenol group must possess a sulfonic acid group. If less than 50 per cent acid is used for the sulfonation process, the resulting product is water-soluble in high concentrations only and precipitates upon dilution. While all of the various sulfonation products precipitate gelatin, only those sulfonated with less than 75 per cent acid can be salted out.

In contrast with this behavior all natural tanning agents can be salted out and, since the ability to be salted out has been accepted as a measure of tanning action, those syntans possessing salting-out properties are considered best suited for tanning purposes. The authors demonstrated this action in subsequent experiments, and thus explained that the tanning action of the

syntans containing sulfonic acid groups is not entirely due to interaction between the sulfonic acid group and the basic groups of the hide substance but largely to the presence of other specific groups in the molecule. They differentiated between auxodepse and depsophore groups. The auxodepse or hydrophilic SO_3H-groups primarily effect the necessary water solubility. When a solution of the syntan is brought in contact with hide substance, the auxodepse groups of the molecule interact with the basic groups of the protein, thereby lowering the acidity and consequently decreasing the hydrophilic character of the complex. The depsophore groups of the now less hydrophilic complex are then enabled to exert their full tanning action.

Although the assumed depsophore group has not as yet been identified, the hypothesis was advanced that it may be a $—C^{+}=C^{-}—$ linkage of a neighboring phenolic hydroxy group.

Observations of this kind led to the production of syntans of a more complicated nature.

Newer Syntans

The phenolic hydroxy groups of the molecules comprising the active tanning substances of vegetable-tanning agents are responsible for their tanning properties; if the hydroxy groups are removed, the tanning properties are removed with them. According to Otto,[3] to possess tanning properties, the phenolic hydroxy groups must be present in a non-dissociated state, *i.e.*, at a pH below 7.0. From investigations made by Felzmann and Otto, it must be concluded that Stiasny is correct when he says the hydroxy groups are not combined with the basic groups of the hide substance by primary valences. He assumes that part of the hydroxy groups is combined with the basic groups of the hide substance by secondary valences, while the larger portion is combined by similar valences with the oxygen of the peptide group of the collagen.

Phenols possessing only one hydroxy group cannot react in this way since their negative charge is too weak. However, with an increasing number of hydroxy groups, the necessary potential can be obtained. By comparative potentiometric titration of vegetable-tannin solutions and of various syntans, Otto investigated their dissociation properties. The results showed that the most important vegetable-tanning agents are half dissociated at pH values between 8.0 and 9.0. Of the syntans only those products which are considered combination tanning agents and exchange tanning agents showed dissociating groups between these pH values. The auxiliary syntans, which possess a strong acid character and whose pH value is around 2.0, show hardly any dissociation in the alkaline pH range.

From these observations, it can be seen that all those syntans which do

not depend solely on sulfonic acid groups for solubility or tanning property, but derive these properties also from an accumulation of weakly acid groups such as hydroxy, carboxyl, chlorine or nitro groups, closely approach the tanning properties of the natural tanning agents.

In order to obtain products of this kind, resorcinol, xylenol, dihydroxydiphenyl compounds, benzidine, chlor-benzols and other aromatic compounds are used, either alone or in combination with phenol or cresol, as a basis for formaldehyde or ketone condensation products. These resinous condensation products are subsequently sulfonated to such a degree that just sufficient water-solubility is assured.

However, even these high molecular compounds do not possess a large enough percentage of aggregated particles. To overcome this deficiency, these products can be recondensed again by themselves or with each other; but all these operations tend to increase the manufacturing cost very considerably. Consequently, products of this kind are made for special purposes only; namely, tanning agents for the production of white leathers of extreme fastness to light.

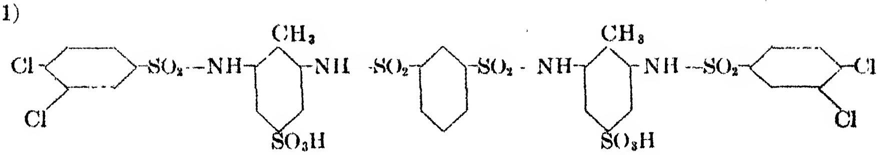
Substitutes for Vegetable-Tanning Materials

For the production of substitutes for vegetable-tanning agents, suitable cheaper raw materials must be used. Natural tanning agents do not derive their particular tanning properties from compounds possessing practically the same chemical constitution, but they are due to a mixture of compounds which are more or less chemically related, but whose molecules are of varying size and in form of a gradated dispersion. Consequently, a syntan of a practically uniform chemical constitution will not produce leather of the same quality as one consisting of a mixture of different condensation products whose complexes vary in size, have the faculty to aggregate, and show in solution as much as is possible a complete gradation in particle size, ranging from the finely divided to a more or less coarse state.

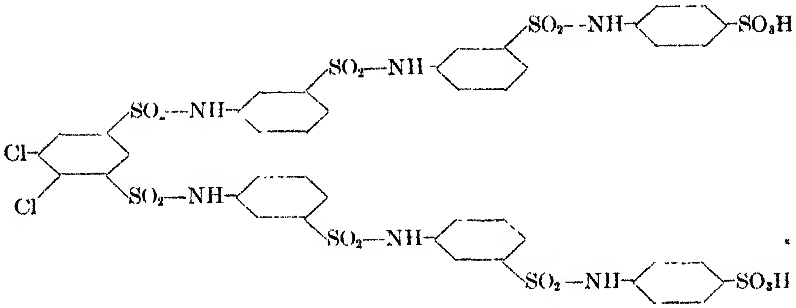
Available natural products most suitable for this purpose are resins, (colophony) tall oil, lignin, cellulose sulfite liquors (spruce extract), etc. These compounds are condensed with aromatic compounds possessing the hydroxy, carboxyl or other acid groups necessary for the required water-solubility and the tanning action desired. If sufficiently water-soluble, they can be used as such; if not, they are carefully sulfonated or dispersed in another suitable sulfonated compound of good water-solubility which may act as a tannin or a non-tannin.

In this way, it is possible to produce syntans which can be employed for practically all purposes. The following theoretical formulas represent the principal constituents of some of the newer syntans.

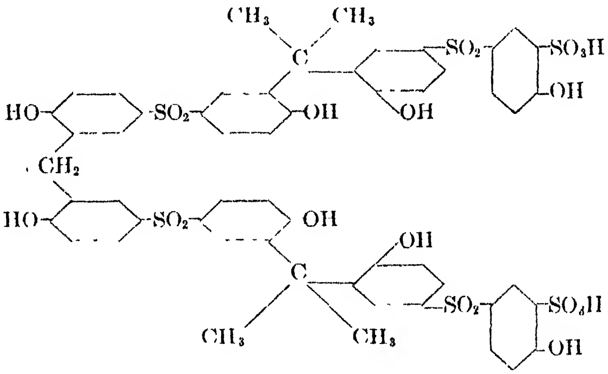
(1)



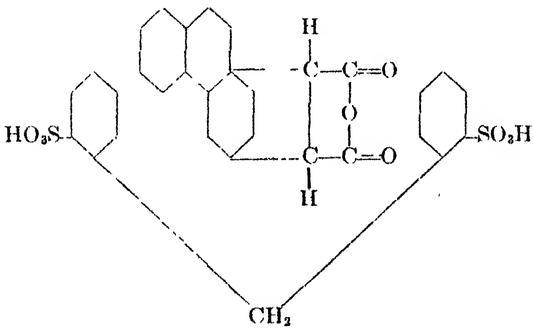
(2)



(3)



(4)



(1), (2), and (3) can be used alone for the production of white leathers of good fastness to light. They can also be combined with compounds similar to (4) in order to impart to them desirable tanning properties. In (4), the reaction product of abietic acid and maleic anhydride is dispersed during manufacture in a benzol sulfonic acid-formaldehyde condensate. In place

of benzol, one of its substituted products, or naphthalene or a naphthalene compound, can be used. In place of the abietic resin of natural origin a synthetic resin (*e.g.*, urea-formaldehyde) can be employed.

Classification of Syntans

The demand for various synthetic products will no doubt increase considerably, particularly when the tanner knows that their use will simplify and speed up the tanning process, and improve the quality of the leather. In order to enable the tanner to select quickly that syntan which is most suitable for the problem on hand, some kind of distinct classification is desirable.

Wolesensky[9] has suggested the establishment of two classes:

"A," Syntans which are manufactured by previous sulfonation and subsequent condensation; and

"B," Syntans manufactured by previous condensation and subsequent sulfonation.

He suggested this kind of classification because of the differences he observed in the tanning properties of the two types of syntans. However, as mentioned before, the sulfonation can be varied in such a way that either manufacturing process yields practically the same end results. Moreover, a class "A" syntan may have been converted into a class "B" syntan by dispersing in it unsulfonated condensation products of gradated solubility and particle size. A chemical classification seems to be out of the question because of the complicated nature of the present syntans. The only distinct classification would be a division into sulfonated and unsulfonated preparations. However, such a classification would be of little value since comparatively few syntans are available which do not possess a sulfonic acid group.

A division into benzol derivatives on the one hand and naphthalene derivatives on the other is also impossible, since either group may contain derivatives of the other groups in the form of a dispersed resin or of a simple sulfonic acid for dispersing purposes or to act as a non-tannin.

If physical means are resorted to, the prospects are more hopeful. Stather and Herfeld[7] found that syntans containing more or less aggregated and dispersed particles show some precipitate in partly or fully saturated salt solutions. It has also been found that strong mineral acids produce a salting-out effect which varies with different syntans. A standardized method based on these observations may be a feasible basis for classification.

The classification established by Otto[4] seems to be the most practical one at the present time. It is based on the comparative dissociation properties in the alkaline pH range of syntans and natural tanning agents. According to this method the syntans can be divided into three classes.

(1) **Auxiliary Syntans.** All products are included which depend for their tanning properties solely on sulfonic acid groups. They are practically strong

acids, show pH values of 1.5-2.0 and dissociate very little in the alkaline pH range.

Their solutions are not affected by the addition of strong acids and very little, if at all, by salt up to the saturation point. They have practically no tendency to aggregate.

(2) **Combination Syntans.** All syntans are included which, like the vegetable tanning agents, possess dissociating groups between the pH range 8.5-9.0 but show a more or less pronounced second dissociation step at lower pH values. This second dissociation is due to the presence of strong negative groups (sulfo).

They are of a considerably less acid nature than the auxiliary syntans and are appreciably more affected by the addition of strong acid and salt. This greater salting-out effect is due to the presence of colloidally dispersed particles having the property to aggregate. The presence of these molecules of larger particle size is the reason for the greater filling properties of this class of syntans.

(3) **Exchange Syntans.** All syntans are included which show dissociating groups between the pH range 8.5-9.0.

They are weakly acid, possess a favorable proportion of tannin to non-tannin, and their ash content is low. The latter condition proves a low content of mineral salts, which makes an unlimited exchange with vegetable-tanning agents possible under all tanning conditions. Mineral acids and salt exert a strong salting-out effect, which shows the tendency of the dispersed particles to aggregate. Since this tendency is equivalent to a simultaneous development of adhesion forces, these poly-dispersed particles, possessing the necessary potential, can be considered the cause for increase in the yield of leather. Some of the syntans belonging to this class combine with the above properties the ability to produce full white leathers of excellent fastness to light.

Depending on the class to which a syntan belongs, it may offer to a greater or lesser degree the following advantages:

Avoidance of case hardening.
Shortening of the tanning process.
More uniform penetration.
Lightening of the natural color.
Increase in tensile strength.
Better utilization of the vegetable tannins.

Application of Syntans

Auxiliary syntans possess a high content of non-tannin and are, therefore, not suitable for the use in vats or handlers. They are used as a pretan or together with vegetable-tanning agents when drum tannage is feasible. Used

as a bleach in place of alkali and strong acids, they prevent bleeding out, supplant excess tannin deposited on the grain surface, and carry it into the inner parts of the leather.

Combination syntans may vary in their composition a great deal. Brands which contain a high percentage of non-tannin are not suitable for application in vats or handlers. Like the auxiliary syntans, they are used as a pretan or a retan, and together with vegetable-tanning agents, for drum tannages. Brands with a low percentage of non-tannin can replace efficiently 25 per cent or more of vegetable-tanning agents; depending on their chemical composition, they exert a softening or a hardening effect on the leather produced. Since they should be used at their natural pH, the skins should be fully delimed before they are exposed to the action of these syntans.

Some brands of this class exert a particularly good solubilizing (dispersing) effect on difficulty soluble vegetable-tanning materials. For this reason, they are well suited to replace a considerable part of the sodium sulfite (or bisulfite) usually applied for solubilizing quebracho extract.

The tanning action of exchange syntans is due to the presence in the molecules of weak negative groups and not of active sulfonic acid groups. The number of these weakly acid groups as well as their dissociation properties compare favorably with those of the natural tanning agents. Consequently, they are absorbed by the hide substance in a similar way and can be exchanged with them in any proportion. Depending on the chemical composition, these syntans impart a soft or a solid feel to the leather and they may tan slowly or rapidly. Their solubilizing action on phlobaphenes is generally good, and most of them prevent the formation of mold in the tan vats.

Summary

Consideration of the data given leads us to classify the syntans broadly into the three following types:

(1) Those depending for their tanning properties mainly on sulfonic acid groups.
(2) Those which depend on the presence in the molecule of weakly acid groups, which in addition to the tanning action, impart water-solubility.
(3) Those which represent a colloidal dispersion of resinous substances in a suitable medium. Those resinous substances, which may be of natural or synthetic origin, possess weakly acid groups in the molecule. The dispersing medium imparts the necessary water-solubility and may act as a supplementary tanning agent or as a non-tannin.

This classification, however, is unsuited for practical purposes, since it does not indicate the tanning action proper.

It appears that only those products are satisfactory substitutes for natural tanning agents which consist of gradated mixtures of various sized molecules. The molecules must possess a sufficient number of weakly acid groups by which they are enabled to combine with the hide substance in a way similar to the action of natural tanning agents.

With the increase of strong acid groups in the molecule, the hydrophilic character of the molecule increases. In consequence of this, the tanning action decreases in the same ratio.

A decrease in the number of strong acid groups increases the salting out properties, thereby illustrating the capability for aggregation and the filling properties resulting from aggregation.

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2. Küntzel, A., and Schwank, M., *Ibid.*, **847, 848** (1940).
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9. Wolesensky, E., Bureau of Standards, Tech Paper No. 309 (1926).

Chapter 22

Miscellaneous Tannages

Niedercorn[8] in a recent article classifies in a unique and comprehensive manner the elements which might be used for tanning, and this classification is therefore given verbatim. "This arrangement of the elements is based upon their electronic structure. Elements having similar properties are correlated together. Chromium is followed by molybdenum, tungsten and uranium. Molybdenum is reduced to the tervalent state with more difficulty than is chromium, but the salts of the two metals are very similar and both tan very well. The molybdenum oxide corresponding to the green oxide of chromium is brown and unlike chromic oxide; it is a strong reducing agent. The molybdic acid corresponding to chromic acid is a feeble oxidizing agent which, when treated with moderately strong reducing agents, forms intensely blue salts. Tungsten is very much like molybdenum, but it is even more difficult to reduce. However, uranium is so different that it forms principally quadrivalent salts, and of course, it is radioactive.

"Since iron is one of the strong tanning agents, we think of that next, and we find that its congeners are ruthenium and osmium, both of them so scarce, that at present they do not promise to be of commercial interest.

"White salts have the strongest appeal, and the aluminum family is one of the most interesting. Unfortunately gallium, indium and thallium are rare, and the last-named is very poisonous. Titanium and zirconium are relatively plentiful, hafnium and thorium are scarce and the latter is radioactive. Zirconium and hafnium are scarcely to be distinguished by their chemical behavior, but titanium differs markedly, and in this difference between the first and the two succeeding members, the chromium and titanium families are similar. Zinc offers some possibilities, but magnesium seems less interesting. Both of these metals have been suggested as tanning agents but were not successful commercially. Cadmium oxides and sulfides are colored, and mercury cannot be considered.

"Cerium has been used in tanning and, since it belongs to the so-called 'rare earths,' all of which have atomic numbers ranging from 57 to 71, its ability to tan is of great interest. All of the rare earths are very similar chemically and some of them are not very rare. Their salts are mainly white, pale yellow, pale red or pale green, so that if they tan, the leathers will be very light in color.

"Scandium and yttrium both form white salts only, but they too are rare.

"Silicon has been used in tanning, but its congeners, germanium (rare), tin and lead are less interesting, since they form highly colored sulfides.

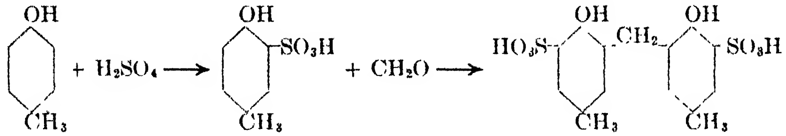
"Aside from the rare earths, the elements potentially most useful in making white leather are probably aluminum, zinc and titanium, followed by zirconium and silicon.

"Zinc sulfate can be used with Tanak MR in a manner similar to that described for alum; it is much less acid than alum, but like alum it causes difficulties in fatliquoring.

"The chemical behavior of zirconium is much like that of titanium, but titanium oxide is much better pigment than zirconium oxide; whether or not titanium leathers will be whiter than the corresponding zirconium tanned leathers is being investigated."

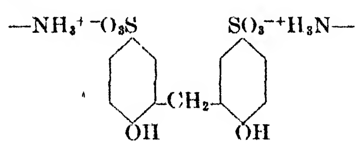
Resin Tannage

The idea of a resin tannage is not new, since many of the so-called synthetic tannages are of the resin type. The materials used for the early "syntans" were sulfonated phenols or cresols condensed with formaldehyde.



These materials are water-soluble and are already polymerized or condensed to such an extent that little additional polymerization occurs during or after the tannage. Many improvements have been made in the production of the synthetic tanning materials, and such modifications are discussed at length elsewhere in this book.

The "Neradol" or soluble-resin type of tannage might combine with the reactive collagen groups in two possible ways, namely through its reactive sulfonic groups or through its active hydroxyl groups. Since this type of synthetic tanning material is most reactive in the acid range, it seems probable that the reaction is by means of the active acid sulfonic groups. This reaction might be pictured:



The above reaction would indicate a bridge formation between two adjacent collagen chains and should therefore result in increased structural cohesive resistance as measured by the shrinkage temperature of the leather

produced. Figure 196 shows the shrinkage temperature of leather made by treatment with two different synthetic tanning materials. One shows a maximum degree of leathering at approximately pH 3.0; the other gives a maximum value at pH 5.0. These results were obtained by Theis and Blum,[15]

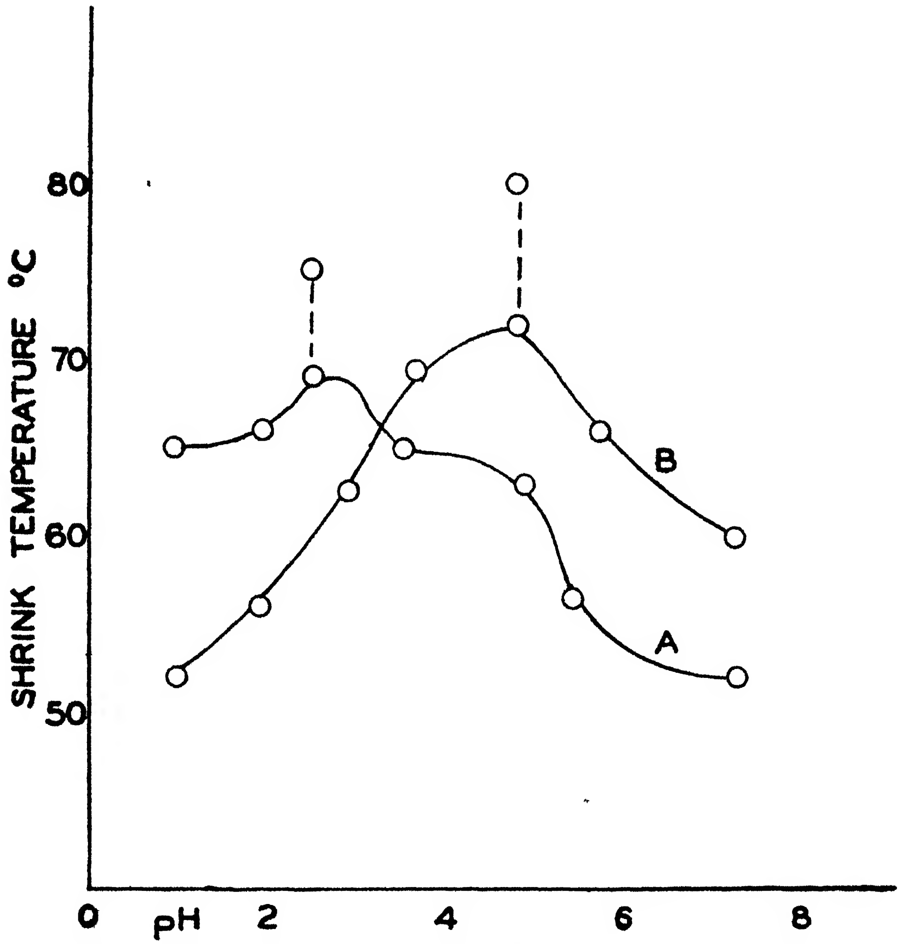


Figure 196. Effect of two syntans upon shrinkage temperature of leathers produced. Dotted lines indicate effect of increased concentration of syntan during tannage.

using a 10 per cent solution of the tanning material, a 48-hour tanning period and a temperature of 20°. The reaction as pictured above is entirely reversible and must in the main be considered similarly to the titration of a protein with an acid having an anion of such a type as to associate itself strongly with the protein by means of several reactive groups.

Theis and Blum[15] have postulated the reaction between the soluble-resin type of "syntan" and collagen as being a linkage of reactive sulfonic acid

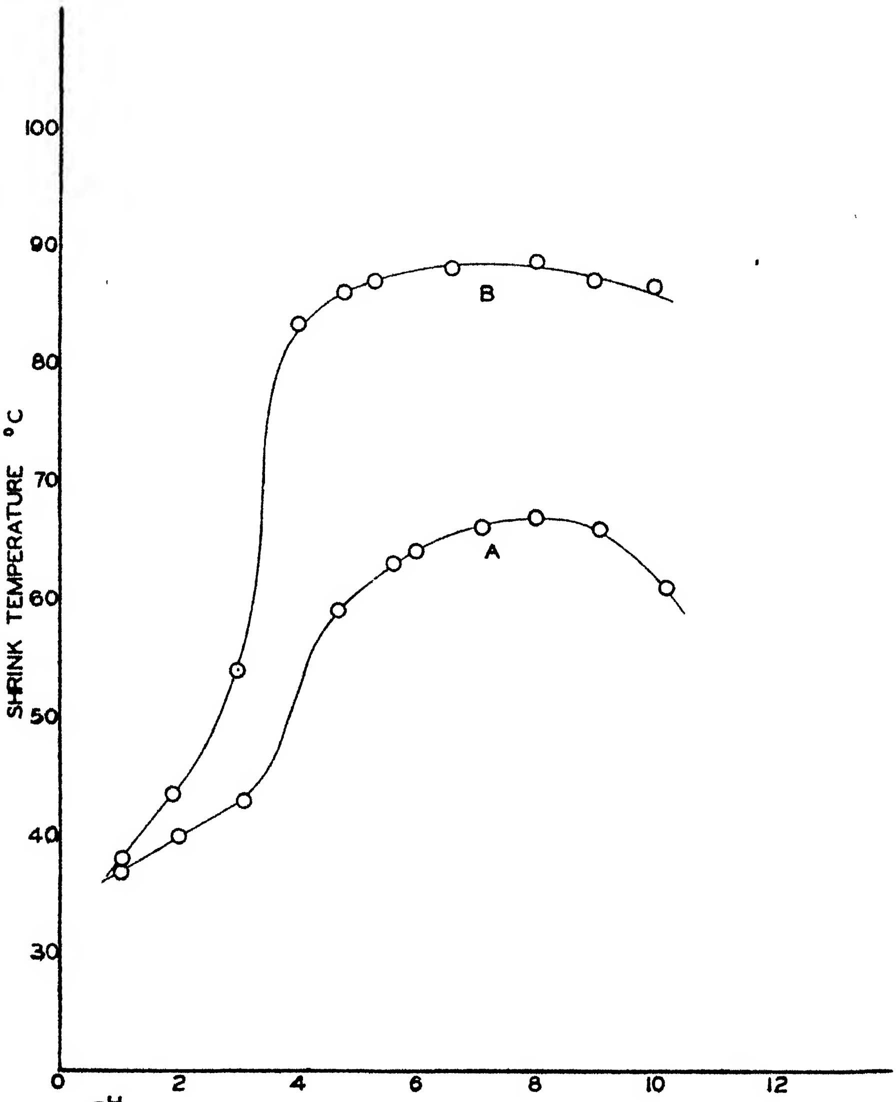


Figure 197. The effect of phenol absorption upon the shrinkage temperature of collagen.

groups with charged basic groups of the protein rather than a reaction of the hydroxyl groups of the "syntan" with the basic groups of the protein. This is indicated when the reaction of phenol with collagen is studied. A 5 per

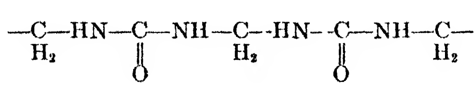
cent solution of phenol adjusted to pH values ranging from 1.0 to 10.0 was used. Constant agitation was maintained over a 48-hour period at 20°. Curve A of Figure 197 shows that in the pH range 5.0 to 10.0, only the natural shrinkage temperature of untanned collagen obtained. However, in the more acid zone, actual structural breakdown was indicated. Curve B of Figure 197 shows the shrinkage temperature of the phenol-treated collagen after subsequent treatment with formaldehyde. Since this particular curve is of the same general trend as that for collagen treated only with formaldehyde, it might be postulated that the phenol interfered in no way with the general and specific reaction of formaldehyde with collagen, *e.g.*, few if any of the reacting groups of collagen, necessary for the formaldehyde reaction, were pre-empted by the phenol.

The real resin tannage of the future is one that is water-soluble and which polymerizes after its penetration into the skin. Whether the subsequent polymerization and condensation within the skin can be classed as truly a tannage is still open to question, but undoubtedly such a process will have interesting application in the leather world.

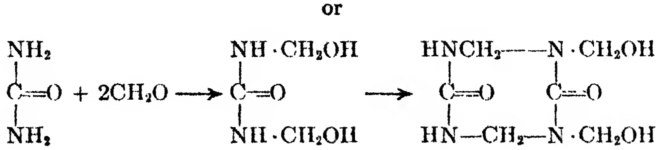
Niedercorn lists the following qualities desirable in such a resin tannage: (1) it must not change too rapidly during storage; (2) it must be water-soluble and its solution should tolerate fair concentrations of salt; (3) it must be capable of penetrating into the skin before and during the early stages of condensation or polymerization; (4) the catalyst necessary for accelerating polymerization or condensation must not injure the skin and it must remain active in its presence; (5) resinification must occur at low temperatures and within a reasonable time; (6) the material must be taken up evenly, *i.e.*, it must distribute itself properly in the skin; and (7) preferably it should be very pale or water-white.

Not just any type of resin can fulfill the necessary requirements and much fundamental research is necessary before the resin type of tannage can of itself be practical.

The urea-formaldehyde resins have been mentioned by Smith;[11] but in this case, the resin was not used as the tanning agent but as an aid in obtaining water resistance. The urea-formaldehyde resins are made by treating urea with formaldehyde. The probable reactions are the following:



or



It is very probable that a mixture of the two types of cross linkage occur. So far as the authors are aware, but little use has been made of this type of resin as a tanning agent.

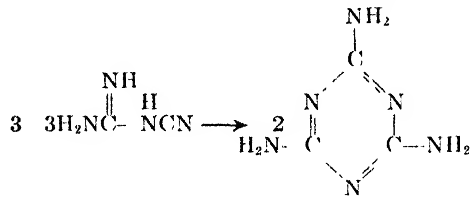
Niedercorn[8] has discussed the use of the melamine-formaldehyde resins in the tanning of leather and has indicated that these materials hold some promise.

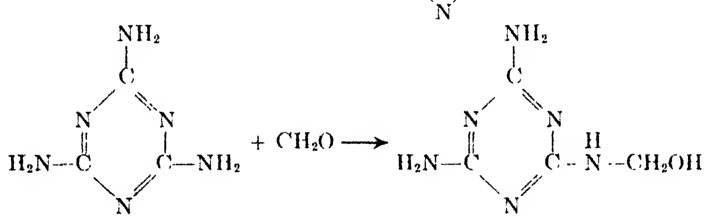
The starting point in the manufacture of the melamine-formaldehyde resin is calcium carbide. The carbide is then converted into calcium cyanamide, cyandiamide, dicyandiamide and then into melamine. The reactions are indicated in the following equations.

$$CaC_2 + N_2 \longrightarrow CaNCN + C$$

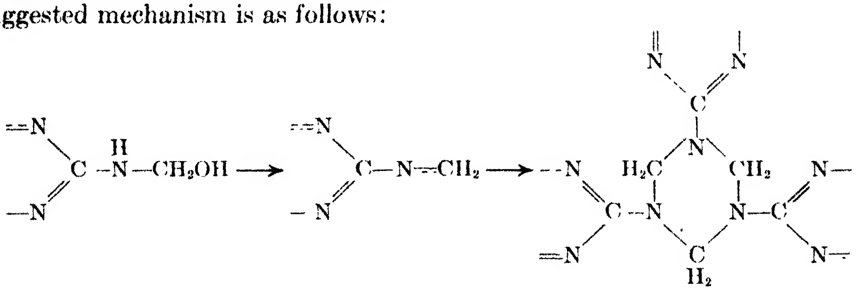
$$CaNCN + H_2O + CO_2 \longrightarrow H_2NCN + CaCO_3$$

$$2H_2NCN \longrightarrow H_2N\overset{\overset{NH}{\|}}{C}-\overset{H}{N}CN$$





The methylol melamines upon acidification condense to white resins and the suggested mechanism is as follows:

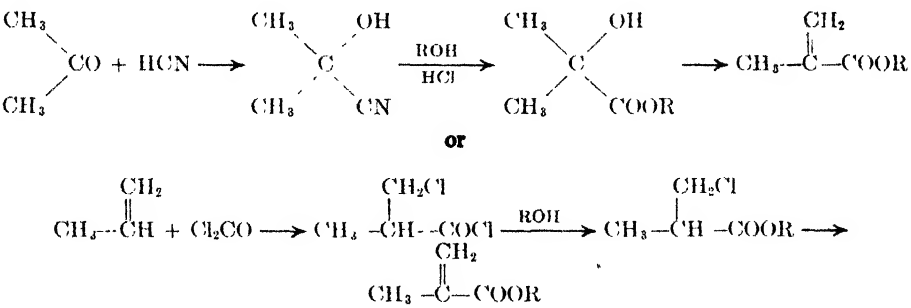


Niedercorn describes melamine as a base, comparable in strength to pyridine but sparingly soluble in water. The trimethylol melamine is described as considerably more soluble and is comparable to aniline in basicity. Due to this basicity, the trimethylol derivative is capable of combining with

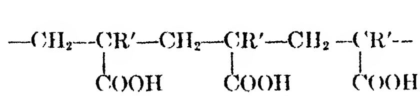
acids, and excessive amounts of acid will keep the resin in solution. Niedercorn recommends two procedures for the use of the melamine resin as a tanning agent, the one to add the resin solution to pickled skin, and the other to add the resin solution to bated or depickled skin. If it is added to pickled skin, care must be taken or complete penetration of the skin will not occur previous to the resin condensation. If it is added to depickled skin, rapid penetration takes place and upon acid addition condensation occurs.

Graves[2] has patented a resin material for the tannage of skin into white leather making use of a polymethacrylic acid. Graves states, "The conversion of skins into leather is accompanied by profound changes in their physical characteristics. They become opaque and a distinct fibrous structure becomes apparent." Graves advocates the introduction into the skin of an acidic polymerization product of methacrylic acid and then decreases the pH value in order to accelerate the fixation. Acidic polymerization products of methacrylic acid react chemically with certain reactive groups of the skin through the free carboxylic groups. Graves defines polymerization in this case so as to include polymerization of methacrylic acid with itself as well as inter-polymerization of the methacrylic acid with other materials.

Methacrylic acid can be manufactured by two methods: the cyanide method and the phosgene method. The following equations illustrate the preparation of methacrylic acid.



By internal condensation the methacrylic acid will form a polymer such as:



The reaction of this type of resin would in all probability be different from that of the melamine resins, since it contains active acidic groups capable of reacting with basic groups of the skin protein. The leather made by the use of the polymethacrylic acid has a high shrinkage temperature and can therefore be considered as an active tanning agent.

Phosphate Tannage

In 1937, Wilson[17] postulated that long chain phosphates would combine chemically with collagen to form stable leather and that the vigor of combination would be increased by lowering the pH value from 5.0 to 2.4.

In his studies on phosphate tannage, Wilson first considered trisodium phosphate. However, since the phosphate anion is negative over a large pH range and has the electron configuration shown below,

```
┌         ..         ┐≡
         : O :
   ..     ..     ..
 : O  :   P   :  O :
   ..     ..     ..
         : O :
└         ..         ┘
```

it becomes a powerful donor of electrons over a wide range of pH values; further, since all the atoms of this anion are electron-saturated, polymerization of trisodium phosphate is not indicated.

Wilson then turned his attention to the simplest member of the series, namely, sodium metaphosphate, which can be represented as follows:

```
   Na
   ..
 : O :
   ..  ..
    P : O :
   ..  ..
 : O :
   ..
```

A compound of this type should readily lend itself to polymerization, since a donor oxygen atom of one molecule may share a pair of its electrons with an acceptor phosphorus atom of another molecule. Molecules of great size might occur in such a system of polymerization.

Polymeric sodium metaphosphate was first studied by Thomas Graham and is commonly known as Graham's salt. It may be produced in the laboratory by fusing monosodium orthophosphate and rapidly cooling the melt. The formula for the cooled mass is generally written $(NaPO_3)_x$.

Wilson's work with the tanning action of the polymeric metaphosphates dealt with a commercial compound known as "Calgon." The analysis of the Calgon glass used by him was as follows:

Na_2O	32.1%
P_2O_5	66.8%
Na_2SO_4	0.2%
NaCl	0.1%

The proportion of Na_2O to P_2O_5 corresponds to a hypothetical sodium hexadecaphosphate, $Na_2O(NaPO_3)_{16}$, but since no such compound is known,

Table 306. Effect of Tanning Time and pH Value of Calgon Flakes Solutions upon the Combination of Phosphate with Cow Hide.

100 lbs bated weight of cow hide treated with 5 lbs Fatliquor in 35 gals water and 100 lbs Calgon Flakes in 73 gals water for lengths of time and at pH values indicated. Values in the table represent parts of constituent per 100 parts of hide substance.

Tanning time in hours	4.00	8.00	12.00	24.00	48.00	72.00
At pH value = 2.5:						
Fat	6.00	7.47	7.91	8.05	11.26	10.74
Water-soluble matter	20.45	24.74	22.39	20.34	23.88	26.32
Total P_2O_5	16.98	18.86	17.70	16.31	18.67	19.75
Combined P_2O_5	5.97	6.12	6.09	5.82	5.75	5.80
At pH value = 3.0:						
Fat	10.09	10.03	10.64	11.30	8.02	7.33
Water-soluble matter	22.45	27.32	25.71	29.73	26.03	30.29
Total P_2O_5	17.01	19.79	20.32	22.00	21.69	19.82
Combined P_2O_5	4.96	4.72	5.41	5.81	5.90	5.67
At pH value = 3.5:						
Fat	8.00	7.50	11.30	9.82	11.47	8.05
Water-soluble matter	19.61	22.06	26.86	22.02	25.60	27.08
Total P_2O_5	13.21	15.57	18.61	15.63	18.28	18.89
Combined P_2O_5	3.05	4.29	4.25	4.20	4.30	4.40
At pH value = 4.0:						
Fat	9.49	10.20	9.97	11.64	12.42	12.77
Water-soluble matter	18.32	20.38	20.24	22.51	25.57	26.70
Total P_2O_5	12.62	13.74	13.99	15.31	17.86	17.45
Combined P_2O_5	2.74	3.14	3.10	3.12	3.34	3.37
At pH value = 4.5:						
Fat	12.27	9.92	10.13	11.01	10.00	9.32
Water-soluble matter	19.08	18.28	20.52	18.44	17.44	21.23
Total P_2O_5	12.06	11.61	13.37	11.82	11.04	14.01
Combined P_2O_5	1.59	1.94	1.96	1.88	1.99	2.03
At pH value = 5.0:						
Fat	10.19	9.23	10.54	12.13	11.77	9.23
Water-soluble matter	20.53	16.77	18.10	21.93	20.76	19.64
Total P_2O_5	11.79	10.01	10.63	12.90	13.12	12.60
Combined P_2O_5	1.13	1.11	1.13	1.19	1.21	1.15

Table 307. Effect of Concentration of Calgon Flakes upon the Combination of Phosphate with Cow Hide.

100 lbs bated weight of cow hide treated for 24 hours with 5 lbs Fatliquor in 4.5 gals water, the indicated number lbs Calgon Flakes in 5 gals water and sulfuric acid in 0.5 gal water to lower pH value to 2.9. Values in the table represent parts of constituent per 100 parts of hide substance.

Lbs Calgon Flakes	1.00	2.00	4.00	6.00	8.00	10.00
Fat	19.10	18.52	14.27	16.45	18.88	9.81
Water-soluble matter	4.79	5.89	7.50	15.42	16.02	14.26
Total P_2O_5	5.27	7.32	9.09	10.05	12.48	11.62
Combined P_2O_5	4.65	5.76	6.34	5.58	5.58	5.62

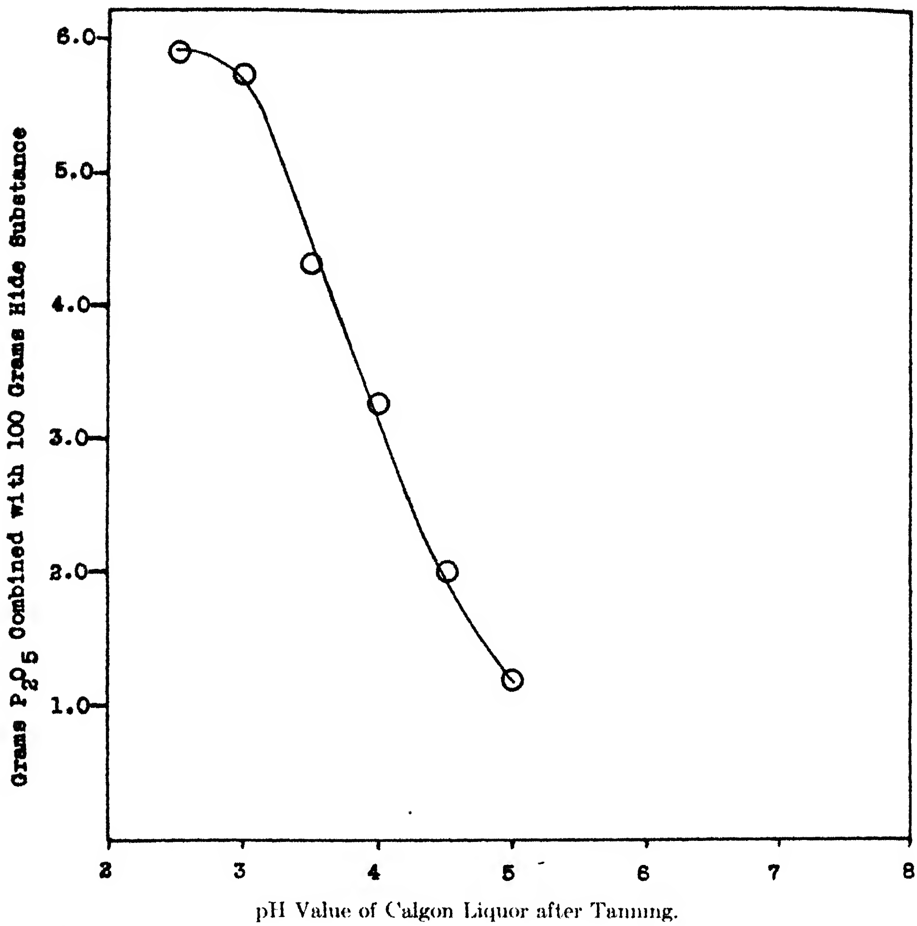


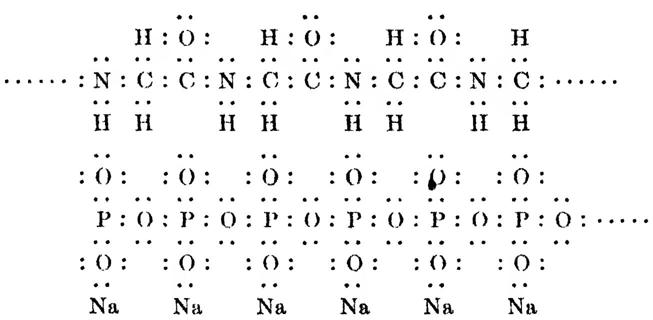
Figure 198. Showing fixation of phosphate by cow hide from Calgon Flakes solutions as a function of pH value

Wilson pointed out that it is more reasonable to consider it rather as a mixture of polymeric sodium metaphosphate and sodium orthophosphate. Wilson pictured the polymeric metaphosphate as follows:

```
  ..      ..      ..      ..      ..      ..
 : O :   : O :   : O :   : O :   : O :   : O :
  .. ..   .. ..   .. ..   .. ..   .. ..   .. ..
  P : O : P : O : P : O : P : O : P : O : P : O : ........
  .. ..   .. ..   .. ..   .. ..   .. ..   .. ..
 : O :   : O :   : O :   : O :   : O :   : O :
  ..      ..      ..      ..      ..      ..
  Na      Na      Na      Na      Na      Na
```

Since the molecule is a large one, Wilson represented the tanning action as

the polymeric metaphosphate linking several protein units together in an electrovalent manner, the vigor of combination increasing with decreasing pH value. Wilson represented the metaphosphate tannage as:



In his studies Wilson investigated the effect of tanning time, pH value and concentration upon the phosphate tannage. His results are shown in Tables 306 and 307 and in Figure 198. These data show the tanning time to be between 8 and 12 hours and the best pH value to be approximately 3.0.

Wilson claimed that the outstanding characteristics of Calgon-tanned leathers are pure whiteness, phenomenal resistance to abrasion and to tearing, and great tensile strength. However, it is well known that skin tanned with metaphosphates dries hard and loses its whiteness. Wilson has pointed out that Calgon-tanned leather has a relatively low shrinkage temperature. Theis and Blum[15] investigated the shrinkage temperature of Calgon-treated skin; they found that the highest shrinkage temperature occurred when the skin was tanned at pH 3.0, but that the shrinkage temperature was rather low; thus they concluded that Calgon was not a complete tanning agent. Their results are shown in Figure 199.

Lindner[6] claims to have been the first to establish the so-called tanning action of polymeric anhydrous phosphates. Küntzel[3] in 1937 noted that polymeric metaphosphates did not give a true tannage when combined with animal skin, in that the leather dried out "horny." Riess[9] found that the use of sodium chloride or sulfate in conjunction with the metaphosphate helped materially in this regard and classified this treatment as being intermediate between an aluminum salt treatment and a true mineral tannage. Riess further found that hide powder absorbed or bound 0.930 milliequivalent HPO_3 per gram of collagen, which is well in line with the acid-binding power of hide powder. He therefore concluded that the combination occurred between the acid and the available amino groups. Lindner criticizes this conclusion, pointing out that not the free acid but an acid salt of the polymeric acid is used, and that a part of the metal is complexly bound in the polymeric phosphate in a non-ionogene form. Lindner studied the tanning action of

the hexametaphosphates. His experimental procedure was the following. The equivalent of two grams of bone-dry, lightly chromed hide powder were shaken for a period of 24 hours with 100 ml of hexametaphosphate solution. The solution contained one gram of phosphate per 100 ml. The pH value of the phosphate solutions was varied from 1.43 to 7.77 with formic acid or

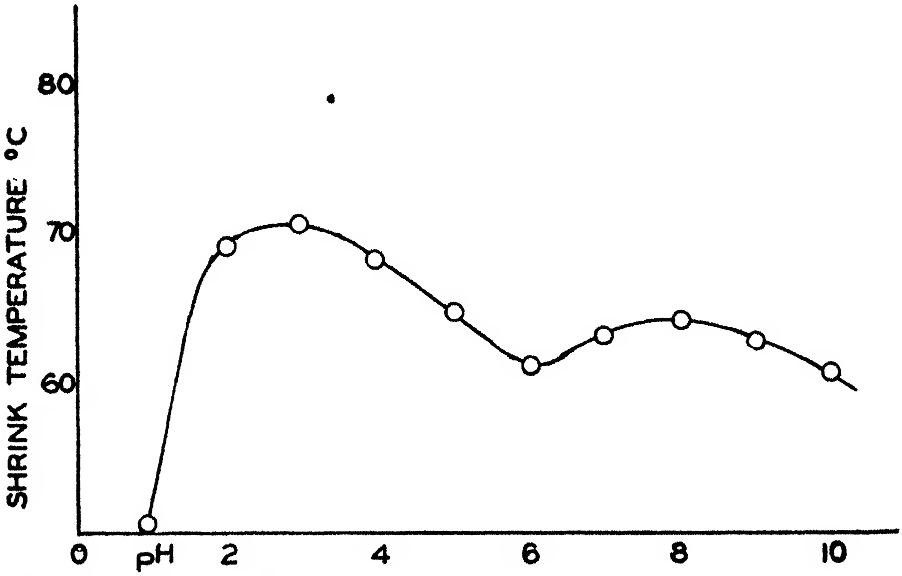


Figure 199. Effect of Calgon tannage upon shrinkage temperature of leather produced.

sodium hydroxide. After the tanning period, the hide powder was removed, washed free of soluble phosphate, dried and analyzed. Lindner's data are shown in Table 308.

Table 308

Tanning pH	Tanning take-up (ash) (%)	P_2O_5 (%)
1.43	1.55	1.05
1.76	1.68	1.12
2.15	2.13	1.38
2.25	2.37	1.51
2.49	3.52	2.42
2.71	2 31	1.47
3 09	1.48	0.98
3.55	1 35	0.57
4.56	0 98	0.35
7 77	4.95	0.12

These data show that a maximum value for tanning, as measured by the absorbed or bound P_2O_5, was obtained at pH 2.49. On either side of this

particular pH value, P_2O_5 binding drastically decreased. In another series of experiments, the hide powder was treated with the hexametaphosphate solution at pH 2.5 for some 20 hours and the pH value was then increased by the addition of sodium hydroxide. An additional 4 hours was allowed for this neutralization reaction to attain equilibrium, and the tanned hide powder was then analyzed. The data are shown in Table 309.

Table 309

Equilibrium pH	Tanning take-up (ash) (%)	P_2O_5 (%)
2.45	3.58	2.38
3.55	1.78	0.62
4.60	1.08	0.40
7.95	5.15	0.13

These data indicate that, when the metaphosphate solution is adjusted toward the end of the reaction, the P_2O_5 fixation is about the same as when the whole period of contact is at that particular pH value. In other words, the neutralization reaction reversed the fixed, water-resistant P_2O_5. In another series of experiments, lightly chromed hide powder was tanned with increasing amounts of metaphosphate at the optimum pH value. These data are shown in Table 310.

Table 310

Metaphosphate used (%)	Tanning take-up (ash) (%)	P_2O_5 (%)
1.0	1.52	0.99
2.0	2 05	1.30
3.0	2.39	1 57
4.0	2.64	1.71
6.0	3.25	2.13
7.5	3 51	2.31
10.0	3 52	2.30
20.0	3.92	2 50
50.0	3 78	2.38

These data show that the P_2O_5 fixation increased up to some 7.5 per cent metaphosphate given and then remained essentially constant. In still further work, Lindner investigated the effect of pH value and constant metaphosphate concentration on unchromed hide powder, with results given in Table 311.

Table 311

Equilibrium pH	P_2O_5 (%)
1.61	2.91
2.09	4.01
2.45	6.37
2.71	5.01
3.25	3.69
4.95	0.51
6.90	0.12

Thus it can be seen that the P_2O_5 fixation increased to 6.37 per cent at pH 2.45, as compared to 2.42 per cent using chromed hide powder, which indicates that even the slight chroming of the hide powder blocked certain reactive groups and lessened the P_2O_5 fixation.

Lindner goes on to state that since the hexametaphosphate must be considered to be an anionic tannage, its reaction with the free amino groups of the collagen supports the auxiliary valence theory of chrome tannage. Lindner made a series of practical tests, but for these experiments used a commercial metaphosphate material known as "Coriagen." Bated calf skins were treated with 5 to 20 per cent of the material in the presence of 0.75 to 3.0 per cent of salt. The tanning liquors were adjusted to pH 2.4-2.5 toward the end of a 6-hour tanning period. After standing overnight, the tanned skins were washed, fatliquored and finished. A very white, thin leather resulted, which had a fine, compact "grain" and contained 2.5 to 3.0 per cent absorbed tanning material, or 1.6 to 1.9 per cent P_2O_5 based upon the dry, degreased weight. In still another series of experiments, Lindner tanned bated calf skins in a manner similar to that already described but finished the tannage at pH 4.0 to 4.2. This leather had a better feel and more fullness than that tanned at pH 2.5.

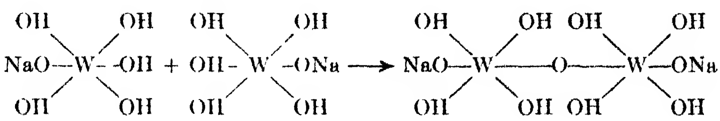
Lindner[6] investigated many different combination tannages utilizing "Coriagen." A coriagen-silica tannage was studied, and it was found that the silicic acid loading gave a fuller leather. Combination phosphate-chrome, phosphate-alum and phosphate-iron tannages were investigated. For the data relating to these various combinations, the reader is referred to the original literature.

In American practice hexametaphosphate tannage alone has not thus far yielded a good commercial leather. Used in conjunction with chromium salts, aluminum salts, formaldehyde, and vegetable-tanning materials, satisfactory leather has been made. Calgon glass can be used as an adjunct quite efficiently in vegetable tanning, since a pretannage with the metaphosphate causes much more rapid penetration of the vegetable-tanning extract into the hide or skin. Commercial use of the material in this way has been accomplished and should be of real interest to the sole-leather tanner.

Tungsten, Molybdenum, and Vanadium Tannage

Küntzel, Riess and Erdmann,[4] and Küntzel and Erdmann[5] have made a study of tungsten, molybdenum, and vanadium polymers as tanning agents. In 1938, these investigators studied acidified solutions of sodium tungstate, Na_2WO_4. They pointed out that, whereas the degree of aggregation of chromium solutions made basic and aged varies continuously, the degree of aggregation curves of acidified polytungstic acid solutions show discontinuities. In the alkaline zone above pH 8.0, mono-tungstate ions are stable and pre-

dominate. In the pH zone 6.0-8.0, a transition zone exists and particles of various sizes appear to occur. At pH values less than 6.0, hexatungstate in the form of an acid salt preponderates. When acid is added to tungstate solutions, free tungstic acid, or an acid tungstate, is formed. After a definite acidity has been reached the acid tungstate ions interact, forming polyacids or white gelatinous tungstic acid. The polymerization of the acid tungstate ions is quite similar to that of the basic chromium salts, namely, two OH groups of two metal complex ions interact:



The pH value of the solution increases because acid groups disappear. The polymerization process is very rapid, and is practically complete at ordinary temperatures in a matter of a few days.

When 1.5 or 1.6 moles of acid are added for each mole of Na_2WO_4 the course of aging is somewhat different, since the acid addition leads to a local excess of acid and thus to the formation of unstable polymers, *e.g.*, $Na(H_5W_6O_{21}$, Aq.), which then depolymerize and gradually form new and more stable aggregates. Possibly even larger aggregates are formed with 12 or more tungsten atoms. When more than 1.6 moles of acid are added, the aging process is again normal. They state that, unlike the basic chromium salts, whose aggregates are of one type and differ only in the size of the aggregate, tungsten polymers of the same size may differ in structure. Again, the chromium aggregate can be changed from a lower to a higher degree of aggregation by simply increasing the basicity. The tungstates do not aggregate to an unlimited extent simply by a change of basicity.

In 1938, Kuntzel and Erdmann[5] further studied the isopoly acids of tungsten. This work was concerned with the establishment of the degree of aggregation which is necessary for a true tanning action. They first studied the precipitation of gelatin by the tungstic acid preparation. Precipitation was taken as a sign of tannage. For their experiments, Küntzel *et al.* stirred a one per cent gelatin solution with an equal volume of acidified and aged $0.1N$ Na_2WO_4 solution. Precipitation occurred as soon as the metatungstate condition obtained, namely, with 1.5 equivalents of acid per mole of tungstate and a pH value of 6.0; but even here a ten fold excess of tungstate solution was necessary for precipitation of the gelatin. A much lower pH value was necessary when equal volumes of gelatin and tungstate solution were employed. These data are shown in Table 312.

Küntzel *et al.* state that the behavior indicated by their data resembles that of vegetable tanning. They further point out that the fact that the

Table 312

Ratio Na_2WO_4 to acid o 1*N* tungstate solution	pH value of solution	Precipitation of mixture containing 1 : 1 gelatin and tungstate solution
1 : 0	7.70	–
1 : 0.1	7.37	–
1 : 0.2	7.25	–
1 : 0.4	7.15	–
1 : 0.6	7.05	–
1 : 0.8	6.97	–
1 : 1.0	6.85	–
1 : 1.1	6.80	–
1 : 1.2	6.68	–
1 : 1.3	6.60	–
1 : 1.4	6.42	–
1 : 1.5	6.00	– (+ 10 fold addition of tungstate soln.)
1 : 1.6	2.70	– (+ 3 fold addition)
1 : 1.7	2.30	+
1 : 1.8	2.05	+
1 : 1.9	1.90	+
1 : 2.0	1.75	+

paratungstate ion with only one acid group cannot precipitate gelatin confirms the theory that a tanning molecule must have not only minimum size but also several points at which salt formation can occur.

Küntzel *et al.* tanned pelt in freshly prepared polytungstate solutions and their aggregation was allowed to occur within the pelt during the two-day contact period. For this study a ratio of pelt to tungstate solution of 1 part to 2000 ml of 0.1*N* solution was used. Their data are given in Tables 313 and 314 and show (a) that with less acid than 1.5 moles per mole of $Na_2WO_4 \cdot 2H_2O$ the pelt is horny upon drying; (b) that in the case of tannage with about 1.7 moles of acid the leather dries white and full; and (c) that with more acid than 1.7 moles the resulting leather shows a tendency to crack. It is stated that the deterioration of the tannage at high acid concentration is due to an acid hydrolysis of the collagen, and this is shown not only by the decreased tannage but also by the reduction in shrinkage temperature.

When pelt was treated with aged polytungstate solutions in a manner similar to that described above, the tanned product was usually hard and partially horny, due to a case-hardening effect. However, if a great excess

Table 313

Mole ratio $Na_2WO_4 \cdot HCl$	Equilibrium pH	Shrinkage Temperature (° C)	Condition after drying
1 : 0.6	7.06	55	horny
1 : 1.0	6.61	55	horny
1 : 1.2	6.16	52	horny
1 : 1.5	5.44	55	horny
1 : 1.7	3.58	69	good
1 : 1.9	1.96	62	cracky

Table 314

Mole ratio Na_2WO_4 : HCl	Equilibrium pH	Shrinkage Temperature (° C)	Condition after drying
1 : 0.4	...	57	very horny
1 : 0.6	...	56	very horny
1 : 0.8	7.70	55	very horny
1 : 1.0	7.36	53	very horny
1 : 1.1	6.94	51	very horny
1 : 1.2	5.91	50	very horny
1 : 1.3	5.54	49	very horny
1 : 1.4	5.25	47	very horny
1 : 1.5	3.63	66	hard leather
1 : 1.6	2.02	68	full-white leather
1 : 1.7	..	62	hard leather
1 : 1.8	..	59	hard, cracky leather

of acid-tungstate solution was used, good leather resulted when only 1.5 moles of acid were used. Employing hide powder, these investigators found that the absorption of tungstic acid increased as the pH value was lowered and reached a maximum value of some 0.4 gram of WO_3 per gram of collagen at pH 2.5. These data are shown in Table 315.

Table 315

Ratio $Na_2WO_4 \cdot 2H_2O$ HCl	Grams take-up per gram hide powder	Equilibrium pH
1 : 0	0.0080	7.38
1 : 1.2	0.1120	6.33
1 : 1.3	0.1380	6.16
1 : 1.4	0.1516	5.90
1 : 1.5	0.2150	5.65
1 : 1.6	0.3084	4.63
1 : 1.7	0.3528	3.53
1 : 1.8	0.3628	2.93
1 : 2.0	0.3952	2.50

Küntzel *et al.* first assumed that the tungstic acid was absorbed as $(H_3W_6O_{21}\ Aq.)^{=}$ and calculated from their data that each gram of pure collagen had bound 0.86 milliequivalent, which is well in line with the values for acid binding. As pointed out previously, Lindner criticized such calculations and expressed some doubt as to whether $(H_3W_6O_{21}\ Aq.)^{\equiv}$ was bound as such; he suggested that a metallic complex was fixed. Küntzel *et al.* then worked with pure metatungstic acid. This solution was found to exert certain tanning properties, but owing to its increased acidity yielded a leather which was hard and brittle. However, it was found that some 0.434 gram of WO_3 per gram of collagen was bound. Upon calculation, this value indicated that 2 equivalents of HWO_3 were bound to each gram of collagen instead of the value expected from stoichiometric relations.

Küntzel and Erdmann[5] studied the phosphotungstic acids along the lines followed in their earlier work. When a solution containing sodium tungstate and disodium phosphate is acidified, the metallic acid first aggregates to the

isopoly acid, or in this case to hexatungstic acid. Several of the aggregates thus formed then further aggregate with the metalloid acid ions. The proportions of phosphorus pentoxide and of tungstic acid in the sodium salts of the various phosphotungstic acid are 1 : 24, 1 : 18, 1 : 12, 1 : 8 and 1 : 6. When a solution of 1 mole of Na_2HPO_4 and 6 moles of Na_2WO_4 is acidified, the crystalline phosphotungstate contains P_2O_5 and WO_3 in the following ratios:

Table 316

pH	Ratio
7.0–5.5	1 : 6
4.5–4.0	1.5 : 12
3.0–2.0	1.5 : 30
1.0–0.0	0.1 : 24

Küntzel and Erdmann[5] prepared the following five crystalline salts; (1) 4-sodium 8-ammonium 2 phospho 1 hexatungstate 2 Na_2O, 4 $(NH_4)_2O$, P_2O_5, 6 WO_3, 2 H_2O; (2) 12-sodium 3 phospho 2 hexatungstate, 6 Na_2O, 1.5 P_2O_5, 12 WO_3, 12 H_2O; (3) 10-barium 3-phospho 5 hexatungstate 10-BaO, 1.5 P_2O_5, 30 WO_3, 73.5 H_2O; (4) 6-ammonium 2 phospho 3-hexatungstate 3 $(NH_4)_2O$, P_2O_5, 18 WO_3, 14 H_2O; (5) 1-phospho 2 hexatungstic acid $H_7P(W_2O_7)_6$ 28 H_2O. One per cent solutions of these salts were used for tanning. Tanning action was shown by compounds (3), (4) and (5) which are rich in hexatungstic acid and are strongly acid in character.

Küntzel and Erdmann[6] studied the tanning action of molybdic and vanadic acids and found that the aggregating powers of these acids were similar to those of tungstic acid. They found that hexamolybdates form in the same pH range in which tungstate ions give hexatungstate ions. Table 317 shows the tanning action of the sodium molybdate solutions used.

Table 317

Mole ratio Na_2MoO_4 to HNO_3	Equilibrium pH value	Shrinkage Temperature (° C)	Condition after drying
1 : 0.8	5.56	59	very horny
1 : 1.0	5.40	58	very horny
1 : 1.1	5.21	59	very horny
1 : 1.2	5.12	61	very horny
1 : 1.3	4.91	60	very horny
1 : 1.4	4.62	64	hard leather
1 : 1.5	4.45	68	full white leather
1 : 1.6	3.95	66	leather
1 : 1.7	3.29	63	leather
1 : 1.8	3.27	61	leather
1 : 1.9	3.28	59	leather
1 : 2.0	2.68	57	leather

These data show that the best tannage occurs at a molar ratio of molybdate to acid of 1 : 1.5. Upon storage the leather changes color, due to the reduction of the hexavalent molybdenum to the pentavalent state.

Table 318 illustrates the effect of acidified solutions of sodium vanadate upon pelt. These data show that optimum tannage takes place at a molar ratio of sodium vanadate to acid of 1 : 1.5. The leather so produced is soft, full, has a light yellow color and a shrinkage temperature of 69°.

Table 318

Mole ratio $NaVO_3$ to HNO_3	Equilibrium pH value	Shrinkage Temperature (° C)	Condition after drying
1 : 0.8	5.56	55	very horny
1 : 1.0	5.40	56	very horny
1 : 1.1	5.21	58	very horny
1 : 1.2	5.12	57	very horny
1 : 1.3	4.91	57	very horny
1 : 1.4	4.62	59	hard leather
1 : 1.5	4.45	69	full white leather
1 : 1.6	3.95	65	leather
1 : 1.7	3 29	65	leather
1 : 1.8	3.27	62	leather
1 : 1.9	3.28	64	leather
1 : 2 0	2.68	62	leather

Zirconium Tannage

Garelli[1] in 1907 made brief mention that the nitrates of zirconium and thallium had some slight affinity for hide powder. This observation was not applied in a practical manner. At one time zirconium was considered to be a rare element but it now appears to be widely distributed in the earth's crust.

The chemistry of zirconium has been investigated quite extensively, and this work is summarized by Venable.[16] Zirconium forms three series of salts, (1) the normal salts such as $Zr \cdot Cl_4$; (2) basic zirconyl salts, such as $ZrOCl_2$; and (3) zirconates such as Na_2ZrO_3. In aqueous solution the normal salts hydrolyze, yielding free acid and basic salts as shown:

$$Zr(SO_4)_2 + H_2O \rightleftharpoons ZrOSO_4 + H_2SO_4$$

In this respect the normal zirconium salts resemble those of chromium and aluminum. However, the zirconium salts hydrolyze to a much greater extent and thus the solutions produced are more acid.

The literature contains some references to the existence of complex zirconium sulfates. Such salts vary widely in basicity from an acid salt such as zirconium dihydrotrisulfate, $Zr(SO_4)_2 \cdot H_2SO_4 \cdot 3H_2O$, to a normal salt such as potassium zirconium tetrasulfate, $Zr(KSO_4)_4 \cdot 3H_2O$, and to a 50 per cent basic salt known as trizirconyl tetraammonium pentasulfate, $3ZrOSO_4 \cdot 2(NH_4)SO_4$.

Somerville[12] in 1942 made a study of zirconium tannage. For this investigation he used the normal salt and was surprised to find that it was taken up by the skin in a strongly acidic state and that it was unnecessary to adjust the basicity by addition of alkali. Of the various zirconium salts available,

the normal sulfate was found to be the most suitable, and was effective both when fully acidic and when neutralized to the basic state $ZrOSO_4$. Somerville[12] employed the normal nitrate, chloride and sulfate and found, after tanning, neutralizing and washing, that the sulfate produced the best leather and the nitrate the poorest.

When a solution of normal zirconium sulfate is added to pickled skin in a brine solution, the pH value of the solution and of the skin drops, but at the same time zirconium sulfate is fixed by the skin. Somerville points out that the basicity of the salt during the early stages of tanning is dependent upon the degree of hydrolysis of the normal salt,

$$Zr(SO_4)_2 + H_2O \rightleftharpoons ZrOSO_4 + H_2SO_4$$

and that this, in turn, is dependent upon the amount of free sulfuric acid present in the pickled skin and in the solution. Somerville found that even with highly pickled skin fixation occurred readily.

Somerville investigated the effect of concentration and basicity of the basic zirconium salt upon its ability to be bound by skin. His results are shown in Tables 319, 320, and 321.

Table 319

% ZrO_2 on pickled weight of skin	Shrinkage Temperature (° F)	Leather quality
1.00	142	partially tanned
1.50	152	partially tanned
2.00	157	fairly well tanned
2.25	168	quite well tanned
3.25	178	well tanned
5.00	188	well tanned and better filled
10.00	206	well tanned and most solid

Table 320

Basicity of solution (%)	0	25	50	75
Tg. after 5 hours*	204	204	188	150
Tg. next morning*	204	204	198	150
pH of exhaust liquor	1.0	1.25	1.75	3.25

* Shrinkage temperature (° F).

Table 321

Basicity of solution (%)	0	25	50	75
Ash	19.0	17.4	18.6	18.3
Combined oxides	18.4	17.1	18.3	18.1
ZrO_2	10.3	10.4	10.6	11.3
Hide substance	58.5	58.5	56.1	57.7
ZrO_2 based on hide substance	17.6	17.8	18.9	19.6

Table 319 shows definitely that a considerable degree of tanning is obtained at quite low amounts of ZrO_2 given, but that for practical tanning greater

amounts should be used; Somerville recommends a minimum value of 5 per cent based on drained pickled weight of skin.

The literature describes several basic zirconium sulfates but, in general, they appear to be of low solubility and tend to separate from solution upon standing. We might picture the formation of these salts as follows:

$$Zr(SO_4)_2 + H_2O \rightleftarrows ZrOSO_4 + H_2SO_4$$
$$2ZrOSO_4 + H_2O \rightleftarrows ZrO_2 \cdot ZrOSO_4 + H_2SO_4$$

Thus on the Schorlemmer scale the zirconium salt $ZrOSO_4$ would have a basicity of 50 per cent, and the salt $ZrO_2 \cdot ZrOSO_4$ 75 per cent. Rodd[10] has reported a still more basic salt having the composition, in anhydrous form, $5ZrO_2 \cdot 2SO_3$. Somerville prepared certain of these basic zirconium salts in which 0, 25, 50 and 75 per cent of the acid present had been neutralized. Tanning experiments were made and his data are shown in Table 321. Somerville points out that it is possible to have a wide range of basicity at the time of application, without affecting the amount of tanning material fixed or the shrinkage temperature of the resulting leather. These data also tend to show that tanning takes place over a wide range of pH values, with no well defined optimum and with uniform fixation at pH values less than 1.75.

In later studies, Turley and Somerville[12] and Somerville and Turley[13] discuss at some length the practical aspects of zirconium tannage. They give as a simplified tannage the following formulation:

Pickled stock	1000 pounds
Water	250 gallons
Salt	100 pounds
Zirconium salt	200 pounds

They suggest that the solid dry zirconium salt should be added to the pickled stock and that the pH value at the beginning of tannage should be between 2.0 and 3.0. The skins should be agitated in this liquor until penetration is complete and then allowed to remain in it overnight before neutralization. They should be neutralized until a uniform pH value of 3.0 is reached, after which they may be washed, shaved and further neutralized to pH 4.5-5.0, when they are ready for fatliquoring.

Turley and Somerville[13] claim that zirconium-tanned leathers have outstanding properties in respect to stability and washability. Such leathers are resistant to hot water, have a satisfactory tensile strength, and are fast to light.

Wilson[17] commented on the studies of Somerville and pointed out that he may not have been dealing with cationic zirconium in the case of zirconium tannage. Wilson postulated the possibility that zirconium sulfate might be a dibasic acid and gave this acid the formula $H^+SO_4^-(ZrO)SO_4^-H^+$.

Wilson stated that if it were a strong dibasic acid its optimum tanning value should then be at a low pH value. He postulates that the two H^+ ions might then combine with two amino groups of the protein and thus link them together by means of an electrovalent link.

Somerville criticized this viewpoint, and pointed out that while zirconium might exist as a zirconium acid salt and combine as such, he doubted if the reaction proceeds in this way, since he had neutralized over 50 per cent of the available acid.

The reaction as given by Somerville is quite different from similar reactions with basic chromium or aluminum salts. It is quite well known that basic chromium sulfate does not bind or fix with protein at pH values much lower than 2.5 and that it can readily be reversed. At pH 1.0 or 2.0 little fixation of chromium occurs and practically all of it can be removed by washing. If, on the other hand, chromium is fixed at pH 3.0 only a small amount can be washed out. McLaughlin[7] and associates have postulated that chrome tanning is in reality the adsorption of acid by the hide protein, with subsequent formation and deposition of 66.67 per cent basic chromium sulfate. They have shown that cationic chrome tanning cannot take place when the skin proteins are acid-saturated. Theis[14] has also demonstrated this fact by an entirely different method. In the case of the so-called alum tanning, higher pH values give not only higher fixation but more stable fixation of the aluminum complex. For this reason, such salts as sodium acetate or citrate are used for the neutralization of the alum liquor. For alum tanning a pH value of 4.5-5.2 is preferred.

Somerville compares the binding of the zirconium compound with the skin protein to that obtaining for chromium and aluminum. It would appear to the authors that zirconium tannage might also be likened to that taking place with tungsten, molybdenum or vanadium, since zirconium fixation requires an extremely low pH value. It would seem that if aluminum, iron, and chromium obey practically the same basic principle when used as tanning agents, *i.e.*, require a relatively high pH value for stable fixation, then zirconium should also obey the same general law if its reaction with skin proteins is similar in nature. The authors in no way dispute the validity of the practical use of zirconium sulfate as a tanning agent, but they question to some extent whether the zirconium is acting wholly as a cationic complex; it would seem that considerable further study is necessary in this regard. The further investigation of the zirconium complex should be interesting and fruitful.

Oil Tannage

Oil tannage has been discussed in the second edition of this monograph. Since we can find no new work in this subject for the past fifteen years, the interested reader is referred to the second edition.

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Chapter 23

Neutralizing

At the completion of chrome tannage the leather must be properly prepared for the next process steps of dyeing and the fatliquoring or stuffing which follows dyeing. This preparation process is termed "neutralizing"; it consists of the removal by washing of any uncombined chrome liquor or neutral salts remaining in the leather, followed by the neutralization with mild alkali of any free acid left in the washed leather and, finally, washing the neutralized leather free of the neutral salts formed in neutralizing. These steps are accomplished in the tannery as follows.

The tanned leather is mechanically pressed or "set-out" as it leaves the tan drum, whereby most of the uncombined chrome liquor it contains is removed. It is then mechanically treated to render it uniform in thickness throughout the entire skin area and to remove any particles of flesh or muscle adhering to its flesh side; these two mechanical processes are termed "splitting" and "shaving." The leather is then placed in a drum and is given a short wash with flowing water, whereby any remaining uncombined tan liquor is removed. The proper amount of mild alkali is now added to the closed revolving drum which is run for the proper period, at the end of which any free acid has been neutralized and the leather is of the proper pH value. The leather is then washed for a short period with running water. The composition, volume, and temperature of the water employed in these several operations and the type and amount of alkali used vary from tannery to tannery, with the type of leather to be produced, and with the desired leather characteristics. For these reasons, it is neither practicable nor necessary to state process details.

But the tanner knows that if any appreciable amount of uncombined chrome or neutral salts is left in the leather, or if it contains free acid, their presence will affect the dyeing, the fatliquoring and the appearance and quality of the finished leather. The desired extent of acid neutralization (that is, of both the free and a necessarily small part of the combined acid) may be determined as a function of the pH value of the leather. This value was formerly determined by means of litmus paper or other indicators, which yielded rough approximations only; when accuracy is desired, the glass electrode is employed. In the latter method, the leather is squeezed by hand

and the pH value of the expressed liquid may be quickly determined. Indicator solutions which cover varying pH ranges are, however, useful to indicate roughly the variation in pH value of the various "layers" of the tanned skin in cross-section.

If nothing more important were involved in neutralizing than the apparently simple factors described above, there would be no point in further consideration of the process. But practical experience has shown that even slight variations of procedure, or changes in the type of neutralizing alkali employed, may have far-reaching effects upon the finished leather. For these reasons, leather chemists have endeavored to explain the mechanism of neutralizing.

When chrome leather is washed prior to neutralizing, changes in its composition may occur, in addition to the mechanical removal of the uncombined matters present. The leather contains protein-bound acid and also acid combined with its fixed chrome. Both forms of fixed acid are partially hydrolyzed and removed when the leather is washed; and when the washed leather is then treated with alkali, both forms of fixed acid may be partially neutralized. The comparative extent to which these various changes occur no doubt has an important bearing on the characteristics of the finished leather. It is quite probable that anions of the neutralizing alkali may penetrate the fixed chrome complex, displacing other complexly held groups and thus radically changing the composition of the chrome complex. Various neutralizing alkalies of the same equivalent strength may differ in the extent to which they penetrate the leather's cross-section. Thus a strong alkali like sodium hydroxide is not employed because it reacts too rapidly with the acid present in the outside layers of the leather, greatly increasing their basicity, and producing undesirable characteristics. On the other hand, milder alkalies, such as borax, sodium bicarbonate and ammonium bicarbonate, react more slowly and more uniformly. Stiasny[5] has recommended the use of ammonia/ammonium salt buffers. These buffers may be adjusted to a low alkalinity so that they act slowly and do not over-neutralize the surfaces of the leather. As the ammonia component of the buffer mixture is decreased, its ammonium salt fraction increases and the alkalinity of the neutralizing mixture decreases. There are other but less employed neutralizing agents, such as sodium silicate (water glass), phosphate, formate, and acetate, as well as certain types of syntans.

In 1929, Schindler, Klanfer and Flaschner[4] cut 100-gram specimens from the butt of commercially tanned calf skin which had been split and shaved. Such specimens were shaken for 2.0 hours at 20° (30° in the case of borax) with 100 ml of water containing the neutralizing agent; the agents included sodium bicarbonate, sodium carbonate, sodium hydroxide, borax, ammonia, ammonia/ammonium chloride and ammonia/ammonium sulfate. The amount

of acid neutralized at the end of the two-hour period was determined and was found to be essentially the same for all the reagents employed. The rate of neutralization was then determined, employing similar experimental methods; the results are given in Table 322.

Table 322

Percentage reagent used, based on split leather wt.	Percentage reagent neutralized, after: 5 min	15 min	30 min	60 min	120 min
1.0 Sodium bicarbonate	74.5	84.0	94.0	94.5	97.0
2.0 Sodium bicarbonate	66.0	76.0	88.5	90.5	94.5
4.0 Sodium bicarbonate	54.7	66.7	76.3	80.3	90.3
4.0 Sodium bicarbonate	48.5	59.2	74.5	80.1	90.0
1.0 Sodium carbonate	67.5	87.5	91.2	98 0	98.0
2.0 Sodium carbonate	61.0	74.0	85.5	94 5	94.5
2.0 Sodium hydroxide	...	98.0	98 0	99.0	99.0
2.0 Borax	..	57.5	86.0	93.5	96.0
2.0 Ammonia	62.0	80.5	87.5	91.5	94.0

Table 322 indicates considerable variation in the speed of reaction of the different reagents. These authors then determined the comparative neutralization of the acid present in the grain and in the middle layers treated with various reagents, after a two-hour treatment, as shown in Table 323.

Table 323

Percentage neutralizing agent	Percentage of acid removed: Grain	Middle
1.0 Sodium bicarbonate	31.6	12.0
2.0 Sodium bicarbonate	44 5	36.4
4.0 Sodium bicarbonate	76.6	52.0
2.0 Sodium carbonate	5.5	19.5
4.0 Sodium carbonate	88.6	50.0
2.0 Sodium hydroxide	74.0	14 6
2.0 Sodium silicate	103.0	21.6
2.0 Borax (30°)	67.2	21.0
4.0 Borax (60°)	87.0	61.5
2.0 Ammonia	27.0	13.0
2.0 Ammonia + 2 moles NH_4Cl	18.0	2.3
2.0 Ammonia + 1 mole $(NH_4)_2SO_4$	33.0	-0.6

In 1934, Riess and Papayannis[3] treated with various neutralizing agents wet chrome-tanned hide powder, containing 3.25 and 5.33 per cent Cr_2O_3 fixed from 33.3 and 50.0 basic chrome alum solutions. These agents included the following compounds: sodium bicarbonate, sodium carbonate, sodium silicate, sodium oxalate, di-sodium hydrogen phosphate, and borax. The wet chromed powder was treated for 30 minutes with the various neutralizing solutions and was then washed with water, dried and analyzed. In this way it was found that not only was the basicity of the leather (*i.e.*, based on total fixed sulfate) increased in all cases, but that an exchange of anions had occurred. That is, the treated leather contained anions of the neutralizing

agent, the amount of which varied greatly with the nature of the agent employed. Since these experiments were conducted with hide powder, we have no way of knowing how direct is their relation to the neutralization of whole leather.

In 1935, Innes[1] neutralized freshly tanned and washed chrome leather with various neutralizing agents, employing 1.25 equivalents of the total fixed sulfate in the leather. The following sodium salts were used: bicarbonate, borate, thiosulfate, tetrathionate, phosphate, silicate, and oleate. Analysis of the treated leather showed a varying degree of removal of fixed sulfate as a function of the kind of neutralizing agent employed; thus the borate removed 91 per cent and the tetrathionate only 32. Analysis of the spent neutralizing solutions indicated a variation in the amount of anion of the neutralizing agent remaining in the leather, which was, for example, 26 per cent of the amount given in the case of tetrathionate and 89 in the case of the silicate.

In a series of papers starting in 1934, Otto[2] has considered the mechanism of neutralizing, more particularly in reference to its influence upon the subsequent dyeing of the leather. He stresses the probable importance of the penetration of neutralizing anions into the fixed chrome complex and suggests, for example, that the alleged difference in the neutralizing behavior of borax and sodium bicarbonate may be explained by the slighter tendency of the borate ion to complex penetration compared with the carbonate ion. Otto performed experiments upon the reaction between various dyestuffs and prepared chrome compounds. He treated a chrome alum solution with NaOH until it was 70 per cent basic and then washed the precipitated basic sulfate until the wash water showed no sulfate ion. The sulfate ion was then so firmly held that it was only very slowly released by continued washing. When this compound was treated for 30 minutes with 0.025 per cent of Cotton Brown A, the dye was completely absorbed and the solution gave a distinct test for sulfate, indicating replacement of complexly bound sulfate. Otto then studied the reaction of dyes with pure chromium hydroxide, which he explains as an anionic interchange; and he made the important observation that pretreatment of the chromium hydroxide with salts of complex penetration ability hindered subsequent dye absorption.

In 1937, Theis[6] studied the neutralizing effects of various compounds. Regular chrome-tanned shaved calf leather was cut into samples of equal area and equal to 5.0 grams of dry leather. This leather was then treated as shown in Table 324, where the percentage of neutralizer given is based upon the shaved weight. At the end of the treatments noted, the pH value of the leather surface and also its interior, was determined by means of indicators. The values shown indicate appreciable differences in the neutralizing behavior of the various agents.

Table 324

Neutralizing Reagent Used	pH Values Surface	pH Values Center
No neutralizing—no washing	4.4	4.2
Washed but not neutralized	4.4	4.4
1% $(NH_4)HCO_3$	6.2	5.2
2% $(NH_4)HCO_3$	6.2	6.2
3% $(NH_4)HCO_3$	7.8	7.0
4% $(NH_4)HCO_3$	8.2	7.6
5% $(NH_4)HCO_3$	8.2	7.6
10% $(NH_4)HCO_3$	8.2	7 6
1% Na_3PO_4	5.6	3.8
2% Na_3PO_4	5 6	3.8
3% Na_3PO_4	6.4	3.8
4% Na_3PO_4	7.2	5.4
5% Na_3PO_4	8.2	7.0
10% Na_3PO_4	9.0	9.0
2% $NaHCO_3$	5.8	5.8
2% Na_2CO_3	6 4	5.2
2% Borax	6.4	5.0
2% NH_4OH	9 2	6.0
2% Sodium acetate	5.4	5.4
2% Sodium formate	4.6	4.2
2% NH_4HCO_3	6.2	6.2
2% Na_3PO_4	5 6	3.8
10% Leukanol	2.0	2.6

Theis then determined the change in the sulfate basicity of the various leathers after neutralizing; this value was based upon the total acid sulfate content of the leather. The results of such experiments with ammonium bicarbonate and sodium phosphate are shown in Figure 200.

Despite the extensive experimental studies described above, we still lack the knowledge necessary for a clear understanding of the actual—and probably the most important—effects of the neutralizing alkali upon the leather. That is, we do not know the relative effect of a given neutralizing process upon the protein- and the chrome-bound acid of leather, nor has any really convincing evidence been offered to prove that the neutralizing anions usually employed actually penetrate the fixed chrome complex, thereby displacing other complexly held groups. Our knowledge of this latter point is based entirely upon analogy and probability.

Until we are in possession of more satisfactory analytical methods for differentiating the various types of fixed acid in chrome leather, we can determine only approximately the relative values of protein- and of chrome-bound acid. But attaining such even approximate values might prove of importance in connection with neutralizing. This could be accomplished by pressing all uncombined matters from leather before and after neutralizing and then determining the protein- and the chrome-bound sulfate in both specimens by means of Gustavson's method described on page 442. This method could be extended to show the comparative depth of penetration of various neutralizers, by splitting the leather into layers, then pressing and

analyzing, a duplicate specimen being neutralized and then split, pressed and analyzed.

A promising method for determining the extent to which neutralizing anions may penetrate the complex of the fixed chrome of leather is as follows. Chrome sulfate-tanned leather is neutralized (after washing in cool flowing

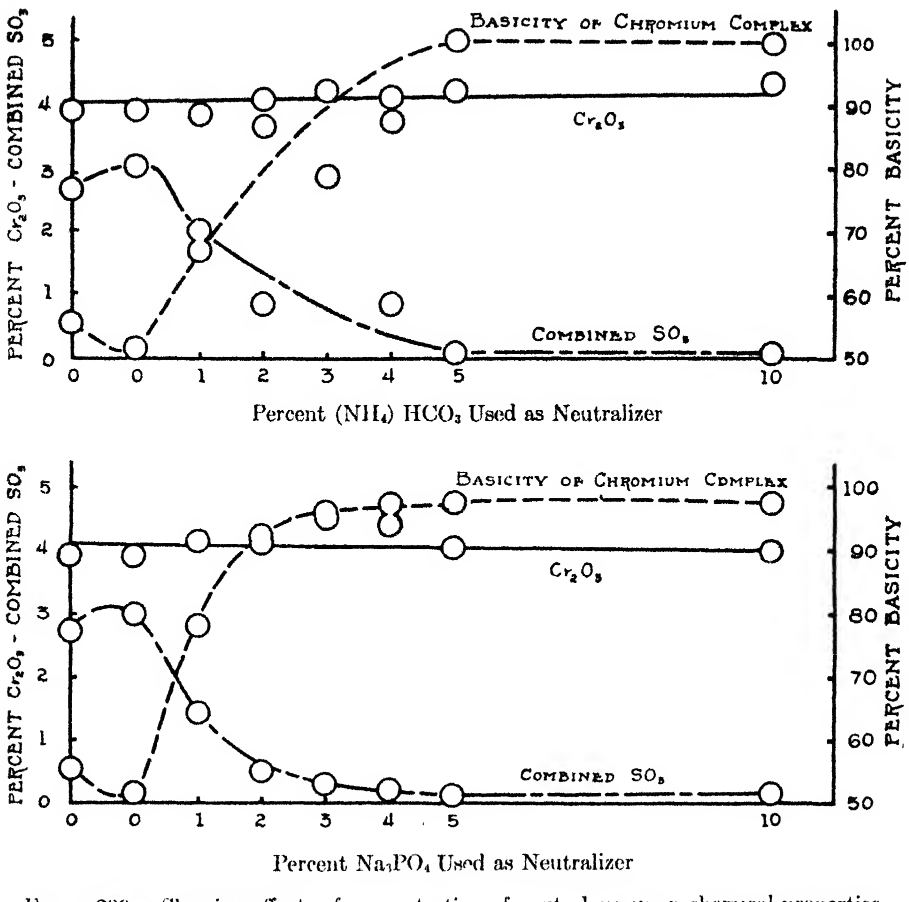

Figure 200. Showing effects of concentration of neutralizer upon chemical properties of the leather

distilled water for one hour) according to Gustavson's method (page 442) until it shows a basicity of approximately 83.3 per cent, when it will contain practically no protein-bound acid and the acid sulfate present will be complexly bound. (The leather should contain around 8.0 per cent fixed chrome, thus insuring that appreciable fixed acid sulfate will be present, or 2.5 per

cent.) Specimens of this leather may then be treated with various neutralizing agents, pressed, and the remaining fixed acid sulfate determined. In this way the actual displacement of complexly held sulfate may be ascertained. Determination of leather shrinkage temperature in future neutralizing experiments will add to their value.

As has been noted above, the neutralized leather is dyed and is then fatliquored. We still lack sufficient knowledge of the mechanism of leather dyeing to permit a profitable theoretical discussion of that subject, although continuation of studies such as those of Otto should help to furnish the needed information. We shall therefore refer the interested reader to works dealing with the practice of dyeing and shall proceed, in the next chapter, to discuss fatliquoring.

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Chapter 24

Fatliquoring, Oiling, and Stuffing Leather

1. Salts of the Fatty Acids (Soaps)

The use of soap as an emulsifying agent for various raw oils in the application of oil emulsions to leather antedates by a considerable time the use of sulfated oils for a similar purpose. For the last fifteen years the published literature on soap emulsions for leather is meager compared to the voluminous and comprehensive literature on sulfated oils.

Soap emulsions are used in some special cases for fatliquoring leather because they have advantages when applied to certain types of leather, especially shoe upper leather such as calf, kid and kangaroo, and also certain types of side leather and garment sheep. The oil dispersion in a soap emulsion is usually much coarser, and also is broken more readily by chrome leather acids or by stripped vegetable tannins than a sulfated oil emulsion. Therefore, soap emulsions do not always penetrate as deep into the leather and do not give the same degree of softness as sulfated oils. All of this means that slightly more oil can be applied to such leather in a soap emulsion without bringing about too great softness and raggedness, thereby obtaining a greater stitch-tear strength than could be accomplished with a smaller amount of sulfated oil. Soap emulsions can, of course, simultaneously fatliquor and neutralize chrome leather, but in doing so they may give a harsher surface feel and a poorer break, in the opinion of many tanners.

Dorey[17] gives a very interesting discussion of the effect of the mode of preparation on the disperion of soap-stabilized emulsions. This appears to be the first publication of quantitative observations which show the kind of dispersion that can be expected from certain methods of emulsification. Dorey made a size-frequency analysis of various preparations and showed the percentage of oil globules of various sizes obtained by different methods. Preparing a 10 per cent olive oil with 0.5 per cent sodium oleate in 89.5 per cent water emulsion with a mechanical mixer, it has been found that the percentage of globules less than one micron in size was 47.5; if the same was prepared with a homogenizer the percentage of such globules was 71.8. When such an emulsion was prepared in a different manner by allowing the soap to form *in situ*, the mixer gave an emulsion with 68.5 per cent of globules less than a micron and the homogenizer 80.7 per cent. Soap formation during

the emulsification process was accomplished by adding the alkali to the water and the fatty acid to the oil phase. It can readily be seen that by the latter method it is possible to obtain almost as well dispersed an emulsion as by using a homogenizer in the usual procedure of adding the oil to the prepared soap solution.

Rayner[47] has reported that when soap is allowed to form *in situ*, very much more effective detergent properties are obtained. An outstanding advantage of the method of allowing the soap to form *in situ* is in those cases where hard fats or waxes are to be emulsified. In such a case the hard fat or the wax is heated until it is completely melted; then the required amount of oleic acid, or other fatty acid, is added and mixed. The required amount of alkali of a suitable concentration in hot water is then added slowly, with vigorous stirring. The mix should be kept sufficiently hot that all the ingredients remain molten. Ammonia is not suitable in such a hot preparation, but either sodium or potassium hydroxides or various amines are suitable. Such a procedure is described by Steinle and Budner[70] in U.S. Patent No. 2,234,934 for the emulsification of carnauba wax.

The proportion of soap to oil in various alkaline fatliquors can vary from 1 : 2 to 1 : 15. A common proportion used is 1 part of soap to 5 parts of oil.

The hard soaps, like the soda soaps (castile soap), etc., can form a spue on leather, especially chrome-tanned leather, as will be considered later. Observations have been made in tanneries where a change to the soft potash soaps eliminated the spue. If splitting of the soap occurs in the leather, then there can be little choice between soda or potash soaps, since in that case the titer of the soap stock is most important. Potassium soaps are better emulsifiers than sodium soaps.

New emulsifiers that are auxiliary agents to soaps have become commercially available, produced by the esterification of the glycols with fatty acids as described by Bennet[5]:

$$\begin{array}{l} CH_2OH \\ | \\ CH_2OH \end{array} + 2CH_3(CH_2)_{16}COOH \longrightarrow \begin{array}{l} CH_3(CH_2)_{16}COOCH_2 \\ \qquad\qquad\qquad\quad | \\ CH_3(CH_2)_{16}COOCH_2 \end{array} + H_2O$$

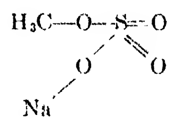
The optimum detergent and emulsifying properties are realized by mixtures of the mono- and di- esters; accordingly "diglycol stearate" is a mixture of the mono- and di- stearates.

2. Sulfated Oils

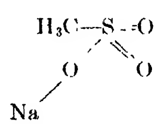
When fatty oils are treated with concentrated sulfuric acid under the usual conditions of careful control, a number of substances are produced but (except in special cases) they include only a very small proportion of sulfonic acid compounds, if any. Therefore, the process should be called sulfation instead of sulfonation. In 1847 Mercer and Greenwood took out a patent

for a "sulphated product" and in 1922 Proctor stated that "sulphonated castor should more properly be called 'sulphated castor.'" Nevertheless, the terms "sulfonation" and the misnomer "sulfonated oils" have been used erroneously in literature all over the world when applied to the sulfuric acid esters of the fatty oils.

In 1939, Koppenhoefer, and at about the same time Burton and Robertshaw in England, made the first major attempts to correct this error and called these esters *sulfated oils* in their extensive publications. These sulfuric acid half-esters are hydrolyzable in boiling dilute mineral acids and are characterized by a sulfur through oxygen to carbon linkage which can be illustrated by the very simple formula of the half-ester of sodium methyl sulfate as follows:



The true sulfonic acids are not hydrolyzable, except in a few special cases, and have a direct sulfur-to-carbon linkage, as shown in the structural formula for sodium methyl sulfonate:



Various types of true sulfonated compounds will be discussed later.

In the words of Schindler, the most important fatliquoring products of the leather industry are sulfated oils, and their general and extensive use in this industry substantiates that statement. It is not within the scope of this treatise to discuss the manufacture of sulfated oils, which has been done by Stiasny and Riess[71] and by Burton and Robertshaw.[13] However, a discussion of the analysis of these oils will be given because of their extensive use in industry and because their analyses have been comprehensively studied during the past twelve years.

There are two phases involved in the analyses of sulfated oils: (1) the commercial analysis for evaluation and preliminary study, and (2) fractionation of these oils into groups of compounds for more detailed study as to nature and properties. The latter phase will be discussed later.

In regard to the commercial analysis, a committee of the A.L.C.A.[46] has investigated various procedures and has come to the conclusion that the methods of the A.S.T.M. presented the most complete and adequate procedures, except that, in common with all other methods available, they did not apply to true sulfonates, which are not hydrolyzable, but did apply to

sulfated unsaponifiables, such as sulfated alcohols, which are hydrolyzable. The old A.L.C.A. methods and those of all other American associations were largely evolved and assimilated from the fundamental work done by Ralph Hart. The Wizöff Society in Europe published standard methods in 1931 that are applicable to oils containing sulfated alcohols. The new A.L.C.A. method for sulfated oil analysis is an adoption of the A.S.T.M. procedures in which the committee referred to above has worked out a modification which makes it possible, in the presence of true sulfonates, to determine the "Total Desulfated Fatty Matter" and the "Unsaponifiables." These methods are also applicable to oils containing sulfated unsaponifiables such as sulfated alcohols.

In regard to the second phase, it has long been realized by various investigators that sulfated oils are very complex mixtures composed of several different and chemically distinct groups of compounds, which fact is often overlooked in considering their use in industry. Stiasny[72] was the first to attempt to separate sulfated oils into their various chemical groups and to study their nature and composition. Schindler and Schacherl[57] extended this fractionation and developed a complicated and detailed separation into seven groups of compounds. This fractionation procedure was later studied by Theis and Graham,[76] who modified and simplified Schindler's procedure somewhat. These investigators fractionated a number of sulfated vegetable oils, also neatsfoot and fish oils, and obtained six groups as follows: (1) free fatty acids, (2) neutral oils, (3) unsaponifiable, and (4) polar, one, two, and three groups. The emulsifying constituents were separated into the three polar groups. This method used the acid value titration, which was considered sufficiently accurate unless the free fatty acids and neutral oils were to be individually studied, in which case the Schindler separation method was recommended.

More recently, Hart[18] published a less complex procedure insofar as only three distinct classes of compounds are obtained: (1) the true fatty oil sulfates, (2) the free fatty acids, (3) and the neutral fatty oil. However, Hart's procedure does not permit the quantitative separation of the three classes of compounds, but does provide the means for the calculation of the factors by which the corrected values of the fractions are obtained. Hart's procedure for fractionation was used by Koppenhoefer in his very thorough investigation of the chemical constituents of sulfated oils, not only because of the simplicity and ease of manipulation, but because Hart's procedure made it possible to separate sufficiently large fractions for extended study later. Koppenhoefer fractionated and analyzed quite completely the various fractions of three types of sulfated oils, *i.e.*, castor, neatsfoot, and cod oils.

Table 325 shows the corrected per cent of the various fractions obtained from the above three sulfated oils.

Table 325

	Sulfated Castor Oil Corrected % Fraction	Sulfated Neatsfoot Oil Corrected % Fraction	Sulfated Cod Oil Corrected % Fraction
Sulfo oil	32.3%	13.9%	18.9%
Free fatty acid	31.6%	55.1%	56.0%
Neutral oil	36.1%	31.0%	25.1%

Table 326. Analysis of the Separated Fractions from Sulfated Castor Oil.

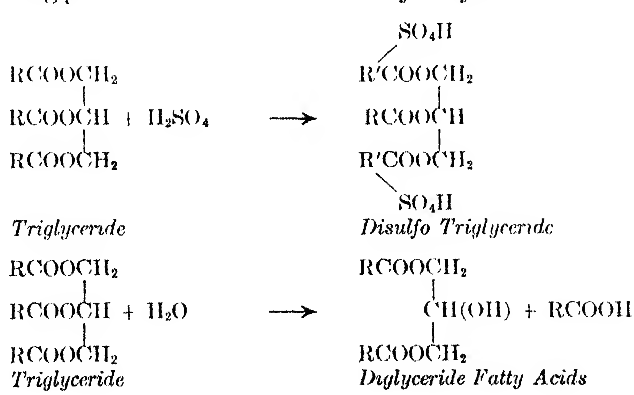
Fraction	Sulfo Oil	Free Fatty Acid	Neutral Oil
Per cent water	18.5	0.0	0.0
Per cent volatile solvents		2.0	1.6
Per cent ash	6.8	0.0	0.0
Per cent total fatty matter	60.5	97.6	97.4
Per cent combined SO_3	11.5	0.09	0.01
Per cent as sulforicinoleic acid	56.5	0.44	0.05
Acid value	27.6	17.9	5.7
Per cent as free ricinoleic acid	14.7	9.5	3.0
Saponification value	176.5	176.9	172.3
Acetyl value (on H_2O-free sample)	131.9	127.1	150.2
Per cent total acids	60.5	93.5	96.2
Iodine value	74.7	75.0	77.6
Mean mol. wgt. (titration)	355.2	313.1	309.0
Mean mol. wgt. (saponification)	306.4	299.0	304.3
Acetyl value	155.7	135.3	134.2
Per cent oxidized acids	59.4	49.9	26.2
Per cent unoxidized acids	36.1	43.6	70.2

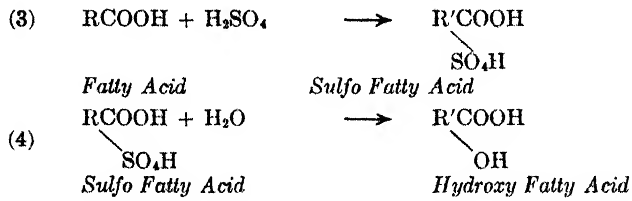
Only a part of one table is given to indicate the completeness of analysis in this work. The most important information brought out about this one oil is that the sulfation of castor oil occurs predominantly at the hydroxyl group; but simultaneous sulfation, to the extent of about 5 per cent, also occurs at the double bond of ricinoleic acid.

Reactions during Sulfation. The groups of compounds formed are partly indicated by Hart[18] in his investigation of the reactions that occur when neutral olive oil is treated with concentrated sulfuric acid in the usual commercial practice. Similar reactions probably hold true for castor oil, except that a larger amount of free fatty acids is formed because of the greater ease with which the triglyceride of ricinoleic acid is hydrolyzed.

(1)

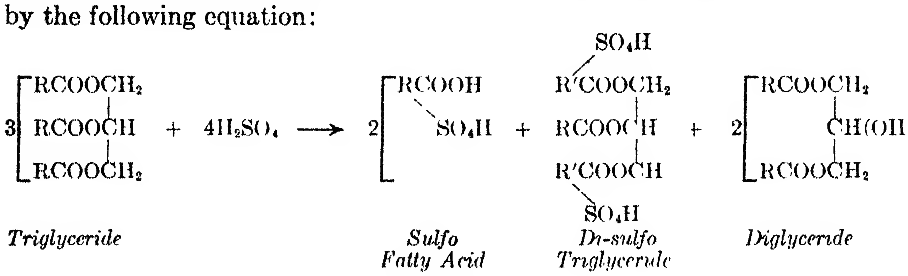
(2)





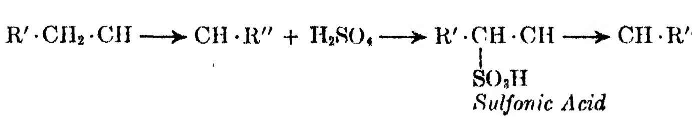
Four reactions seem to take place, namely, the triglyceride is sulfated, yielding a disulfo triglyceride, probably symmetrically arranged; one-third of another molecule of triglyceride is hydrolyzed, yielding a diglyceride and free fatty acids; the free fatty acid is sulfated, giving sulfo fatty acid; and, finally, part of the sulfated glyceride is decomposed to yield the corresponding hydroxy compound.

These reactions, with the exceptions of the partial and total hydrolysis of the glyceride and the decomposition of the sulfo oils, may be represented by the following equation:



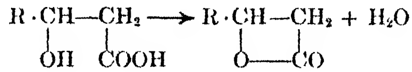
The above equations that Hart gives may represent substantially the reactions that take place during the sulfation of olive oil under carefully controlled conditions; however, it is generally well understood in the sulfated oil industry that the sulfation of more highly unsaturated oils than olive, such as fish oils and particularly cod oil, is accompanied by reactions far more complicated than those given above, resulting in various oxidation, polymerization, and condensation products that are extremely difficult to separate and therefore not fully understood. It is also generally believed that the lower the temperature at which sulfation takes place, the fewer and simpler are the reactions.

Burton and Robertshaw[13] extend very considerably a discussion of the possible reactions beyond those given by Hart. Aside from the sulfonation of aromatics, they show the possibility of formation of true sulfonic acids by combination with any of the double bonds in a fatty acid or by combination at the CH_2 group adjoining one of the unsaturated CH═CH groups as follows

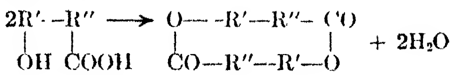


Burton and Robertshaw[13] also show the possibilities of lactone, lactide and estolide formation as follows:

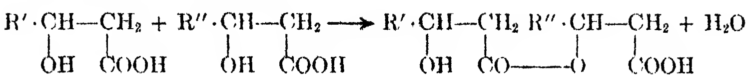
(a) A molecule of a hydroxy acid may lose a molecule of water, giving a lactone or inner anhydride:



(b) Two molecules of a hydroxy acid may condense with loss of two molecules of water, giving a lactide:



(c) The COOH group of one fatty acid may react with the OH group of another acid, giving an estolide:



(d) One molecule of water may be removed from two molecules of hydroxy acid, or two molecules of water may be removed from three molecules of hydroxy acid, and so on, giving complex estolides which saponify with difficulty.

(e) Formation of highly oxidized or polymerized or condensed products of unknown constitution which are probably formed during sulfation to a greater or less extent because some oils contain constituents which are insoluble in water or petroleum ether.

Riess[48] has shown that iso-oleic acid is formed during sulfation of oils. An ordinary neatsfoot oil contained 1.1 per cent of iso-oleic acid; however, after sulfation at 25° with 20 per cent H_2SO_4 the sulfated oil contained 9.3 per cent of iso-oleic acid with a melting point over 50°.

Physical Examination of Sulfated Oils. *Types of Emulsion.* An ordinary commercial sulfated oil may give either a solution or an emulsion with water. Such an emulsion may be either transparent, translucent, or opaque. The degree of opacity may vary considerably and far beyond the power of the human eye to discern by mere observation of a 5 per cent or 10 per cent emulsion. This great variation in types of emulsion is somewhat independent of the particular oil sulfated, but it is to a large extent dependent on the degree of sulfation and to a considerable extent dependent on the subsequent treatment and the neutralization. Any of the above types of emulsions may be suitable for fatliquoring some type of leather. In general, the more opaque types of emulsion are considered to be taken up better in fatliquoring. Nephelometric comparison of various emulsions or comparison with a

standard can be done with a lactoscope, or better with a photoelectric turbidimeter.

Methods for determining emulsifying power of a sulfated oil are important if the oil is to be used as a carrier or emulsifier in a compounded product. Burton and Robertshaw[13] describe a procedure for the determination of emulsive capacity.

3. Petroleum Sulfonic Acids

The petroleum sulfonic acids which are produced during the acid refining of petroleum distillates are sometimes used, in various purified and neutralized forms, as emulsifying, stabilizing, and wetting-out agents in the treating of leather. Investigations of petroleum sulfonic acids have resulted in a division of these products into "mahogany acids" and "green acids." Schestakoff gives the empirical formula for the "mahogany acids" as $C_nH_{2n-12}SO_3$. These acids are generally used in the form of their sodium salts, usually referred to as "mahogany soap." Burton and Robertshaw have outlined a procedure for the analysis of these petroleum sulfonates, the combined SO_3 of which can be determined after fusion with an alkali and oxidizing agent.

Other Fatliquoring Materials (4 to 20)

4. Napthene sulfonic acids (sulfonation products of the cyclic aliphatic substances). These have the general formula $C_nH_{2n-11}SO_3H$, the alkali salts of which have emulsifying and detergent properties.

5. Sulfonated alkylated aromatic hydrocarbons, of which sulfonated dilaurylnaphthalene is an example.

6. The sulfuric acid half-esters of the fatty alcohols constitute one of the important groups of sulfated products that are used in the leather industry. Sulfated lauryl and myristyl alcohols are examples but other fatty alcohols are also used. Balfe[2] reports that catalytic hydrogenation of unsaturated fatty acids can be so controlled that the carboxyl group is reduced to an alcohol group permitting the formation of long-chain alcohols which retain the unsaturated linkages of the fatty acids. The sulfate esters of these alcohols form a series of compounds which have exceptional wetting-out properties. An example of a sulfated alcohol that is used as a dispersion or penetrating agent either before, during, or after fatliquoring is Gardinol.

7. An example of a condensation product of a fatty sulfonic acid is oleic amido ethane sulfonic acid.

8. An example of sulfonated ether is sulfonated lauryl phenyl ether, which is one of the Tritons.

9. An example of a sulfation and condensation product of aliphatic acids having masked carboxyl groups is oleic amido ethane sulfuric ester.

10. Bases such as amino, ammonium, and pyridine compounds and similar substances.

(a) A class of auxiliaries recently introduced comprises the so-called "cation-active" materials. Chemically, these products are salts of relatively strong organic bases. They ionize in solution to give an organic or inorganic anion and an organic cation of high molecular weight which determines the surface activity of these products. The structure of the cation determines whether the material will have "softening," "wetting," or "emulsifying," properties. According to Roth,[50] practically all the cation-active materials in use today are either salts of tertiary amines or quaternary nitrogen compounds. Somerville[65] describes the groups, tertiary alkyl amines of the type

R′ R H

N--R and the tertiary amine salts of the general formula R′—N—Cl,

R′ R″

where R is a long-chain alkyl of at least 8 carbons and R′ and R″ are short chain alkyls of not more than four carbons or of substituted alkyl groups;

R R‴ R R‴

also the quaternary amines such as R′--N or the salts as R′—N

R″ OH R″ Cl

This patent also describes the possible uses of these materials as emulsifiers for oils in fatliquors. Commenting on these claims, it would appear that if leather when immersed in water takes on a positive charge, as Wilson[81] claimed and as Atkin and Thompson[1] stated, then it would be expected that these cationic substances would not be taken up and fixed by leather like the anionic sulfated oils. However, because of their inert reactivity toward the positive leather they would be expected to penetrate much more easily and rapidly than any of the anionic materials and therefore give good softness and good tensile strength to the leather.

(b) The behavior of these bases to the dyes ordinarily used in leather presents a difficult problem. They are inert to the basic dyes but are incompatible in a solution with the acid dyes. These cation-active emulsifiers are precipitated with alkalies and are solubilized and stripped by acids, which is exactly the opposite to the behavior of acid dyes. These cation-active emulsifiers are not affected by strong solutions of aluminum sulfate nor by the usual metallic soap-forming metals. They are also unaffected by strong mineral acids except, of course, that such mineral acid may alter the nature of the tertiary or quaternary ammonium salt. In some cases, they can be salted out by strong solutions of sodium chloride.

11. Wilson[84] introduced phosphated oils which were produced by the action of phosphorus pentoxide on hydroxylated fatty oils. These oils were claimed

to give additional filling and combining power with leather, especially vegetable-tanned leather, and were recommended to be used with sulfated cod oils and moellons.

12. Jaeger[26] has investigated aqueous emulsions containing esters of polycarboxylic acids with mono- or polyhydric phenols. The esters may contain one or more SO_3H groups, and in the case of the polyhydric alcohols one or more of the OH groups may be etherified.

13. There is an enormous number of patents granted for emulsifying agents, especially in connection with emulsions for use in textile, leather, and similar industries. Clayton[15] states that the conception of polar and non-polar molecular groupings is the basis of modern research on synthetic emulsifiers. However, there is a very large group of natural colloids which act as emulsifiers, the enumeration of which is beyond the scope of this review. The alkylated celluloses are some of the newer emulsifying agents described[15] as having possibilities in the leather industry. If methyl cellulose is dispersed in hot water, it will gradually go into solution as the water cools. This has many possibilities, and if such an alkyl cellulose is put through an homogenizer with various oils or lipids, they will form white emulsions that are somewhat stable to salt, acid, and alum.

14. Most of the gums used for emulsifying agents are the natural gums that are well known and have been used extensively in leather. Since some of them like karaya and ghatti gum up so very quickly in water and form lumps, it is generally best to wet them in alcohol or glycerol and then use cold water with vigorous stirring.

15. Some of the vegetable oils that have come into general use in recent years are teaseed oil, mustard seed oil, rice bran oil, peanut oil, and soybean oil. Some oils, because of war conditions, are unavailable for use with leather, like olive oil; some are scarce, like rapeseed oil, sesame, etc. Cottonseed and corn oils are still being used.

(a) Some of these vegetable oils are so high in iodine value and so low in natural antioxidants that they have to be protected by the addition of synthetic or natural antioxidants.

(b) Efforts have been made to stabilize vegetable oils by replacing part of the fatty acids by aromatic acids.[55]

16. An elaborate review was made by C. E. Bills[7] of the chemical literature on cod liver oil, citing 90 papers in the bibliography. This oil was originally obtained by direct steam-rendering. The stearine is removed from the crude product by chilling and filtering. The occurrence of 17 fatty acids in cod liver oil has been reported; the presence of 9 of these is reasonably well established, the remaining 8 being doubtful. All the known acids fall into a broad series ($C_nH_{2n-2x}O_2$) where n has a value between 14 and 22 and x is the number of double bonds, which may range from 0 to 6. The oxidation

and consequent rancidity of cod liver oil is an autocatalytic process accelerated by the formation of organic peroxides and retarded by foreign substrates.

17. A description is given[55] of three groups of synthetic oils: (1) Esters of purely artificial origin, *e.g.*, butyl phthalate, used as a softener in lacquers, and di-*n*-hexyl phthalate and diamylfumarate, which are said to be valuable additions to fatliquor oils. (2) Esters containing residues of higher natural alcohols, *e.g.*, diethylglycoldiphthalate which increases light stability of leather and hinders spueing. (3) Stabilized vegetable oils referred to above. Aside from the above description of synthetic esters, there are a number of somewhat smaller structures that have been found of value in oils used for fatliquoring such as the propyl, butyl, and amyl esters of palmitic, oleic, and stearic fatty acids. These esters do not have the "body" of neatsfoot oil but are very fluid, of low cold test, and penetrate well.

According to Gnamm,[6] cattle tallow is a mixture of triglycerides of stearic, palmitic, and oleic acids. Monoglycerides are present in minute amounts. It is possible to fractionally crystallize dipalmitolein (M.P. 48, S.V. 202.7, I.V. 30.18); dipalmitostearine (M.P. 55, S.V. 202.2); distearopalmitin (M.P. 62.5, S.V. 195.56); and stearopalmitolein (M.P. 42, S.V. 195.0, I.V. 29.13) from cattle tallow, which contains about 21 per cent palmitic, 50 per cent stearic, and 29 per cent oleic and other mixed fatty acids.

18. Egg Yolk. It was formerly thought that lecithin and other phosphatides were the most important constituents in egg yolk (whole egg) for treating leather, but recently this role has been assigned to the protein. Sell, Olsen and Kremers[63] consider that the most important constituent is an unstable complex compound of protein and lecithin. Hevesi[21] notes that egg oil varies greatly in composition; one oil contained 81.8 per cent oleic, 9.6 per cent palmitic and 0.6 per cent stearic, while another oil contained 40 per cent, 38 per cent and 15.2 per cent, respectively. There are many egg yolk substitutes, most of which contain soybean lecithin, a mixture of phosphatides and various cholamine derivatives. These various substitutes usually take into account the tanner's demand for stability to alum. A number of patents refer to materials such as lecithin, gums, glycerine, glycerophosphates, proteins, soaps, aluminum hydroxide, oils, sulfated oils, etc. The practical use of egg yolk is discussed under the subjects, emulsions and fatliquoring.

19. Moellons, Degras, and their substitutes. There is some confusion among tanners in regard to these terms, as for example, wool grease or moellons are often referred to as "degras." However, the manufacturers of leather oil products are quite clear in their use of these terms and the nature of the material that each term designates. "Natural moellon" is a by-product of chamois tanning. "Artificial moellons" are products of the transformation of

marine oils; (a) oxidized or blown oils (b) partly sulfated or hydroxylated (c) and/or treated with water-in-oil emulsifiers. "Degras" is a mixture of moellons, oils, fats, and oxidized fatty acids, according to Thuau and Lisser.[80] However, "degras" is the French name for fish oil that has been used in chamoising.

Thuau and Lisser[80] made an extensive investigation of moellons and degras, and list 18 formulas for moellon substitutes.

20. Oil and Fat Refining. The trend in oil and fat refining is described by Silman[64] in which vacuum and steam distillation methods for de-acidifying oils and fats are discussed. Bleaching of tallow is accomplished by salt or hypochlorite. Palm oil is bleached with dichromate. Oils should be refined before hydrogenation and halogen and sulfur compounds removed.

One of the most important steps in refining oils for use on leather is simply cold pressing, which will remove stearines and other high melting point fats, thereby giving lower pour and cloud points and making such an oil more suitable for general use in the leather industry. Orthmann *et al.*[43] describe improved methods for determining pour and cloud points.

The Theory and Behavior of Emulsions

Fatliquors are emulsions, and the theoretical treatment of emulsions forms an important section of colloid chemistry.[54] Moreover, the aqueous dispersions of emulsifiers form predominantly colloidal systems. As the systems are concentrated the ions present coalesce, forming colloidal particles; the concentration at which this occurs is the critical concentration. The aggregates are presumed to arise because the molecules are not spherical, but elongated or thread-like. The aggregation phenomena are important because only those materials are emulsifiers, wetting agents, or detergents which aggregate in high concentrations. The aggregation occurs primarily at the interface between the body to be emulsified or wetted and the liquid, and not at all, or only slightly, in the aqueous dispersion. According to McBain,[55] the state in a solution of a colloidal electrolyte (which includes soap) can be depicted thus:

$$\begin{array}{lccl} \text{(II)} & \text{Undissociated simple salt} & \rightleftarrows & \text{Undissociated aggregated salt} \quad \text{(III)} \\ & \uparrow\downarrow & & \uparrow\downarrow \\ \text{(I)} & \text{Simple ions} & \rightleftarrows & \text{Aggregated ions} \quad \text{(IV)} \end{array}$$

the particle sizes increase from (I) to (IV). The degree of aggregation tends to increase with increase in concentration and with the number of carbon atoms. Small particles are the most mobile; hence any alteration in the degree of aggregation affects surface activity. The great importance of particle size in a fatliquor emulsion was emphasized by McLaughlin[31] in his discussion of Koppenhoefer's review of the role of lipids.[29] Schiaparelli[51]

stated that the particle size of suitable emulsions is visible under a microscope and was found to be 10^{-4} mm. He states that larger particles are absorbed on the surface only, while smaller particles are also undesirable because they are easily washed out of the leather.

Commenting on the above statement, the reviewer has found by actual investigation that particles having the size 10^{-4} mm are invisible, and that such emulsions would vary from translucency to transparency. The best optical microscopes are capable of resolving particles having the size $10^{-4} \times 2$ mm, or $\frac{1}{5}$ of a micron, that is, $10^{-3} \times 0.2$ mm. However, by the use of ultraviolet light this resolution can be increased to include somewhat smaller particles. An emulsion in which about 90 per cent of the particles are about $\frac{1}{5}$ micron is opaque; such an emulsion represents the average fatliquor in common use. In the whole field of fatliquoring leather, a wide range of particle size emulsions is used, from considerably less than 1 micron up to about 6 or 7 microns. The latter would be represented by poorly prepared soap emulsions. Blockey, *et al.*[8] investigated the mechanism of the penetration of oil into leather during fatliquoring and demonstrated microscopically the existence of fat globules in freshly fatliquored leather. The predominant size of the globules in the fatliquor used was about 1 micron. On drying the leather the globules disappeared. They also showed penetration into the leather of pigment particles of about 5 microns in diameter.

The properties of *dual emulsions* have been studied and described by Cheesman and King.[14] The term "dual emulsion" is applied to emulsions of the same pair of liquids, with the same emulsifier, in opposite types. Oil-in-water and water-in-oil emulsions were prepared with the pairs H_2O-AmOH and H_2O-kerosene, with various emulsifying agents such as soaps, sulfated and sulfonated oils, etc. In most cases the stability of the unusual form of the emulsion was of the same order as that of the usual form. These results throw doubt on the rule that a given emulsifying agent is capable of stabilizing an emulsion of one type only.

In some cases where take-up of oil is difficult, attempts have been made to break sulfated oil/water emulsions during the course of fatliquoring by various means and procedures, such as lowering pH value, use of salts, inversion of phase, coagulants, and by the use of electrolytes of opposite charge. Some of these procedures may work in rare cases, but in general they are not practical. In some few cases, the actual sulfated oil may be more soluble if the pH is lowered. Salts are not satisfactory because the leather will absorb them. The inversion of phase as, for example, an oil-in-water emulsion to a water-in-oil emulsion, is not practical in a fatliquor. The use of coagulants offers by far the best possibility as an aid to take-up. Egg yolk, albumins, flour, emulsified lecithin, gums, and alkylated cellulose present possibilities.

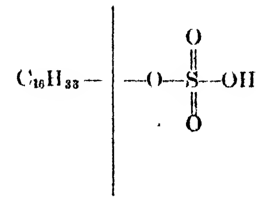
Merrill[35] found that the addition of whole egg to a sulfated neatsfoot oil fatliquor "causes an enormous increase in the size of the oil droplets." The effect of this will be discussed under application of oil emulsions to leather. The use of cation-active tertiary or quaternary ammonium compounds of the fatty acids to break emulsions of the anion-active soaps, or sulfated oils, is very definitely not practical. If such procedure should be attempted, the two opposite electrolytes would precipitate one another, forming a gummy, sticky, resinous mass that would deposit on the surface of the leather, causing some difficulty. This has been observed by the reviewer.

There is generally a normal and gradual break of the emulsion during fatliquoring because the anionic emulsifier is taken up rather rapidly by the leather, causing the remaining emulsion to be unstable and thus bringing about a satisfactory take-up. Therefore, it is usually unnecessary to resort to any of the auxiliary agents referred to above in order to bring about a reasonably complete take-up.

The preparation of emulsions is important and generally fairly well understood by tannery operators. In the Bergman-Grassmann-Gnamm "Handbuch"[6] a description is given for the preparation of a sulfated oil/raw oil emulsion which is similar in procedure to the preparation of a soap/oil emulsion. They say that the sulfated oil should first be mixed with water (boiling water in the case of soap) and the raw oil then stirred in. This is definitely wrong, because the sulfated oil loses its power to disperse a raw oil if the former is first diluted with an appreciable amount of water. The raw oil, whether it be mineral, animal, or other material, *e.g.*, liquid waxes, must first be stirred into the sulfated oil as is, and well mixed; then any dilution can be made. Of course, a solid sulfonated or sulfated emulsifier must first be dissolved in a small amount of water before the raw oil is stirred in.

Soap emulsions are best prepared in boiling water; however, such a high temperature almost always breaks a sulfated oil emulsion. Sulfated castor is about the poorest carrier for other raw oils. One hundred parts of a good sulfated oil, like sperm oil or cod oil, will carry and emulsify about 40 parts of a fatty oil, or about 65 to 75 parts of a mineral oil. Adjustments may have to be made with either alkali or fatty acids, or both, to bring about the maximum carrying capacity of a sulfated oil. On the other hand, soap has a greater faculty of emulsifying fatty oils than mineral oils.

Clayton[16] suggests that lipophile-hydrophile balanced emulsifiers could be used to advantage in fatliquoring leather. He mentions palmityl sulfate as an example of such a balanced emulsifier where the palmityl chain $C_{16}H_{33}---$ is sufficiently, but not too strongly, lipophilic to overbalance the hydrophilic sulfate group, as follows:



The Application of Oil Emulsions to Leather

The most common way of applying oils and fats to leather is in the form of fatliquor emulsions, and the possibility of using any particular material for fatliquoring depends not upon a single dominating property but upon a harmonious blend of many properties. The important considerations are:

During Fatliquoring

(a) Attainment of the desired emulsification.
(b) Accomplishment of the desired fat absorption and distribution.

In the Leather

(a) The effect on the feel in general, the softness, stretchiness, elasticity, and fullness.
(b) Influence on the grain, break, surface feel, etc.
(c) Influence on the flesh and its appearance.
(d) Unalterability of the fat on the fiber.
(e) Influence on the color.
(f) Influence on finishing.

The Mechanism of Fatliquoring

(a) The general methods in common use are: (1) Drum fatliquoring of chrome leather is usually carried out at a temperature of 130° to 140° F for 30 to 60 minutes, and at a concentration varying from 0.5 to 8 per cent of the emulsion. The amount of oil used on basis of the drained wet weight of stock varies from about 1 to about 20 per cent, depending on type of leather. For vegetable-tanned leather the temperature is usually from 115° to 122° F, whereas the other factors are about the same as for chrome leather. (2) Paddle-vat fatliquoring is slightly less efficient because of the very much larger volume of water used, and therefore slightly more oil on weight of stock is required. Because of the large volume of water, the concentration of oil usually varies from 0.25 to 2 per cent of solution and the time of paddling is commonly from 1½ to 4 hours. The temperature is the same as given above for chrome and for vegetable leather respectively, although if the paddle vat is not covered the temperature drops very rapidly.

The application of oil emulsions to leather in still vats, with occasional stirring, is seldom resorted to, and is not satisfactory.

(b) The splitting of sulfated oils during fatliquoring. It was formerly thought that the sulfuric acid esters were split during fatliquoring. However, the works of Schindler and Schacherl,[57] of Burton and Robertshaw,[11] and of Schindler and Römer[60] all show that no noteworthy splitting occurs during fatliquoring within the normal pH range.

Schindler and Klanfer[59] have studied the behavior of sulfated oils during the fatliquoring of chrome leather and found that sulfate is removed from the leather, and that the amount removed increases with the increase of pH value, whereas the splitting diminishes. Sulfated oils have a great power of replacing sulfate, which faculty is also possessed by the true sulfonic acids and by some dye acids. These investigators came to the conclusion that it is very improbable that splitting occurs during fatliquoring.

(c) The process of fat absorption by leather from fatliquor emulsions has been investigated in various aspects by several experimenters. Considering that in some cases chromed hide powder was used, and others chrome-tanned skins, and also considering the variety of materials worked with, it is no wonder that in some cases divergent results and views were reached.

(d) The take-up with chrome-tanned hide powder is much more rapid and complete than with chrome-tanned skin. Stather and Lauffmann found that with unneutralized chromed hide powder the fatty matter absorption from soap solutions and soap/oil emulsions was almost complete up to quite large amounts of added fatty matter. On the other hand, Schindler, Flaschner and Klanfer,[61] using chrome calf skin, showed that with soap solutions the fat absorbed is proportional to the amount of soap used only up to a much lower proportion of fatty matter than in the case of sulfated oils. It would appear that the disagreement is due to the quicker absorption of the hide powder. With sulfated oil liquors and neutralized chrome leather, using practical amounts of oil, absorption is practically complete in two hours, according to Schindler, Flaschner and Klanfer.[61] As mentioned above, it is seldom that tanners will find it necessary in practice to run a mill more than one hour.

The Extent of Fat Take-up

The extent of fat take-up from the emulsion must be regarded as a separate mechanism from that of penetration into the leather; however, both are affected by the proportion of oil to leather used, the concentration of the emulsion, the particle size or degree of dispersion, pre-treatment of the leather, and auxiliary agents used, as for example, egg yolk. The latter material is of unusual interest because of its general use (not only on alum but extensively on chrome-tanned leather) and because of the diversity of opinions

on the effect of egg yolk expressed by various investigators. Stather and Lauffmann[67] found that egg yolk was poorly absorbed by unneutralized chrome hide powder, but the addition of neutral oil increased the per cent of added fat absorbed. Theis and Hunt[74,75] also found that chrome calf skins absorbed fat poorly from emulsions containing egg yolk and that additions of egg yolk to sulfated neatsfoot oil emulsions diminished the absorption,

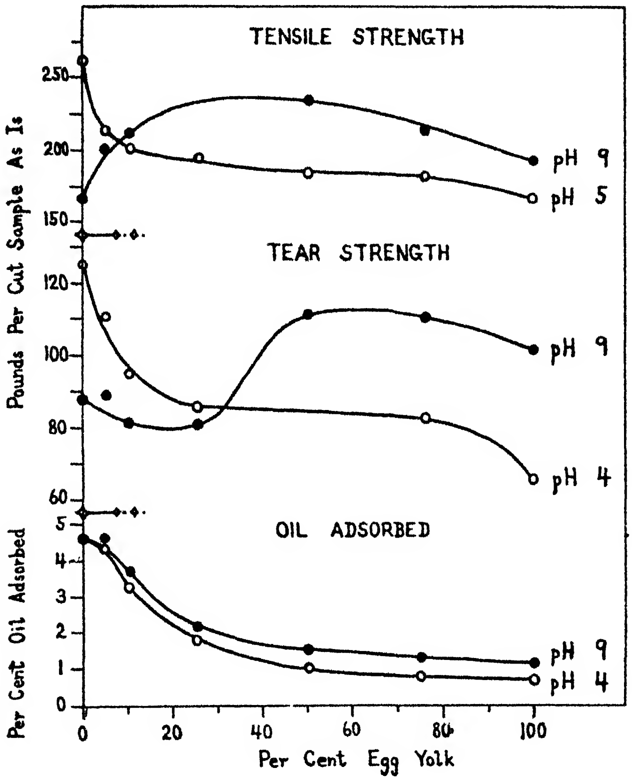


Figure 201. Effect of addition of egg yolk to sulfated cod oil in fatliquoring chrome-tanned calfskin (Theis-Hunt).

as shown in Figure 201. This figure also shows effect of egg yolk on tear and tensile strength and the effect of pH, which will be discussed later. Merrill[35] studied egg yolk but showed principally its effect on distribution. The reviewer has found many tanners who believe the addition of egg yolk favors take-up. It is apparent that it clarifies the fatliquor and that it gives a drier, or a less oily and greasy feel, to the surface of the leather.

Increase of temperature diminished the absorption from soap, sulfated

castor oil, and mineral oil/soap emulsions, according to Schindler, Flaschner and Klanfer.[61]

The influence of variations in pH value of the emulsion has been studied by several investigators. Stather and Lauffmann[66] found that alteration of pH had but little effect on fat absorption; however, Theis and Hunt[75] found a considerable dependence on the pH value of both leather and emulsion on fat take-up. Merrill and Niedercorn[37] found that less fat was absorbed by chromed calf skin from sulfated neatsfoot oil emulsions the more the leather was neutralized. However, in another case Theis and Hunt found, with an emulsion of a mixture of sulfated neatsfoot and raw neatsfoot oil, the optimum value for fat take-up at a pH value of 4.0.

Influence of Pre-treatment of Leather on Fat Take-up

Presumably the whole previous history of the leather to be fatliquored has an influence on the fat take-up. Only two steps in the tanning process have been scientifically investigated in this respect: the chrome tanning and the neutralization. Stather and Lauffmann[67] showed in their investigations of the behavior of hide powder that the chrome content of the hide powder and also the kind of chrome liquor used for tanning exerts no noteworthy influence on the extent of fat take-up. On the other hand, the fat take-up by weakly chromed hide powder was considerably more intensive than by unchromed hide powder. Concerning the effect of neutralization, Merrill and Niedercorn[37] have observed in investigating chrome calf leather that more fat is taken up from emulsions of sulfated neatsfoot oil when fewer acid groups are removed from the leather, which "take-up" was independent of the pH value. Also the kind of neutralizing agent proved to be unimportant. According to Schindler,[54] these statements are partly contradictory to practical experience as well as to the findings of Theis and Hunt[74,75] and the suggestion is made that possibly the observations of Merrill and Niedercorn[37] hold for fat take-up from the emulsion itself, but not for the so practically important distribution of the fat in the leather, which certainly is not independent of the pH value and the kind of neutralizing agent.

Distribution of Fat in Leather

The first investigations of Merrill[35,36] covered the most important fundamentals, namely: (1) the deposition of fat in fatliquoring occurs predominantly in the outer layers; (2) increase in the amount of fat leads finally to penetration into the interior of the leather; (3) pH changes, if they influence the degree of dispersion in the fatliquors, are of importance for the fat distribution. Further investigations of Merrill proved the importance of egg-yolk additions to fatliquor emulsions. It was shown that egg-yolk additions noticeably influence the ratio of the amount of fat taken up by the flesh and

grain layers (Figure 202). As a result of some previous work, Wilson[81,82] emphasized in the second edition of this monograph that the presence of an unfatted middle layer determined the quality of a leather with respect to its firmness, fullness, and strength. However, in 1934 Wilson[84] revised his theories and admitted that an entirely fat-free interior causes poor smoothness, poor break, and other harmful effects on such leather. Merrill found that drying does not influence the distribution of oil in the leather, which is contradicted by Schindler;[53] furthermore, it seems to be generally well under-

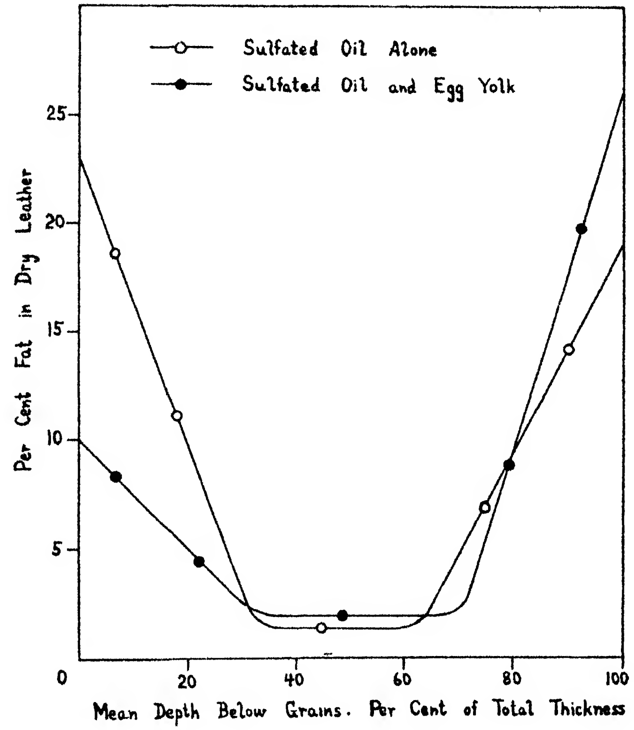


Figure 202. Effect of egg yolk in the oiling of leather (Merrill).

stood in the trade that there is a considerable movement of oil (especially neutral oil) into the leather during the course of drying. More recently Koppenhoefer and Retzsch,[30] in their thorough and comprehensive study, have demonstrated that there is a considerable movement and redistribution of fatty matter during the mechanical operations such as flexing and staking.

Highberger and Moore[22] demonstrated that two types of movement of lipids in opposite direction occurred in heavy vegetable-tanned leather during the first rough drying, *viz.*, (1) movement of natural hide fat from interior to

grain surface producing stains, and (2) movement of added oil taken up by the wet surface layers from the oil wheel, toward the interior of the leather.

Penetration of Fat into Leather

This subject has received unusual attention and interest by many investigators during the past fifteen years, and has a direct bearing on the effect that fatliquoring has on the quality and characteristics of the resulting leather. The earlier works of McLaughlin and Theis[32,33,34] indicated that the various horizontal divisions of a hide or skin presented a distinctive deposition and arrangement of the natural fatty matter. This appears to have laid the foundation for later intensive research concerning the nature and amount of fatty deposition in the various horizontal layers brought about by the process of fatliquoring.

Strather and Lauffmann[67] considered the distribution of fat where chrome calf leather was split into three approximately equal layers after fatliquoring with sulfated castor and neatsfoot oil. They found the distribution to be as follows: grain, 19 per cent; middle, 5 per cent; and flesh, 76 per cent of the total fat absorbed. However, Wilson, Merrill and Daub[82] in 1927 were the first to demonstrate that in fatliquoring, more oil is absorbed and remains in the outer layers than in the middle layer of chrome calfskin.

Theis and Serfass[79] utilized the primary fluorescence of oils under ultraviolet light in a study of the penetration into skin of the oils used in fatliquoring and oiling off. These workers showed the value of ultraviolet light for the microscopic study of the distribution of natural and added fat in an animal skin From their work it appears that the added fat in a sample of fatliquored, chrome-tanned calf skin has penetrated approximately one-fifth of the total thickness into both grain and flesh layers, and that this penetration was more irregular on the flesh side.

Henry[19] investigated the fat distribution in chrome leathers fatliquored with sulfated cod oil and found that: (1) the total absorbed fat is nearly independent of the degree of sulfation; (2) low sulfation of the oil results in better penetration of the leather by all constituents of the fatliquor; (3) sulfated oils as well as neutral saponifiable fats and mineral oils, diffuse into chrome leather by replacement of the water lost during drying.

On the one hand, the affinity for chrome leather is increased by higher sulfation; which favors fixation and enriching of the surface, while on the other hand by higher sulfation the oil dispersion is finer; this acts in the direction of increased penetration into the leather. In the present case the influence of the affinity increase predominated.

The Combination of Oil Fractions in Leather

The subject of penetration of fatliquor constituents into the various horizontal layers and the distribution and fixation of fatty matter in these divi-

sions was also extensively studied by Theis and his co-workers and later by Koppenhoefer and Retzsch. Theis and Graham[77] studied the fatliquoring of chrome-tanned calf skin with various mixtures of sulfated oils and raw oils. In the case of sulfated cod and raw cod oil they found that the portion of combined fat in the grain layer averaged higher than in the leather as a whole, and also that a portion of the neutral cod oil was combined. In the case of mixtures of sulfated and unchanged neatsfoot oil, they found quite

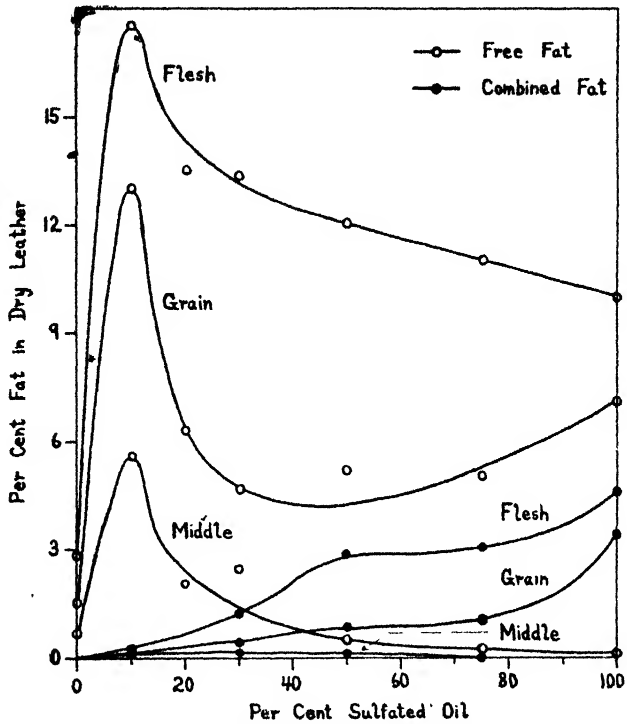


Figure 203. Free and combined fat in chrome-tanned calfskin fatliquored with sulfated Neatsfoot oil-raw Neatsfoot oil mixtures (Theis-Graham).

different results, in that the grain layer had a smaller proportion of combined oil and also that there was no combination of the neutral neatsfoot oil with the leather. Figure 203 shows the percentage of free and combined fat in the dry, chrome-tanned calf skin fatliquored with sulfated neatsfoot oil/raw neatsfoot oil mixtures, according to the findings of these investigators.

Koppenhoefer and Retzsch[30] made a thorough study of the problem of fatliquoring chrome-tanned calf skin with various sulfated oils and their fractions. Mixtures of sulfated, cod, neatsfoot and castor, with their respec-

tive raw oils and fractions were used in this study. They arrived at the following conclusions. (1) The sulfated portion of sulfated fatty oils—the sulfo oil—functions only as an emulsifying agent in the fatliquor and does not assist in the lubrication of the leather. (2) The sulfo oil fraction combines readily in the leather to become solvent-insoluble, and the combination of this chemical group accounts, almost entirely, for the combined oil of

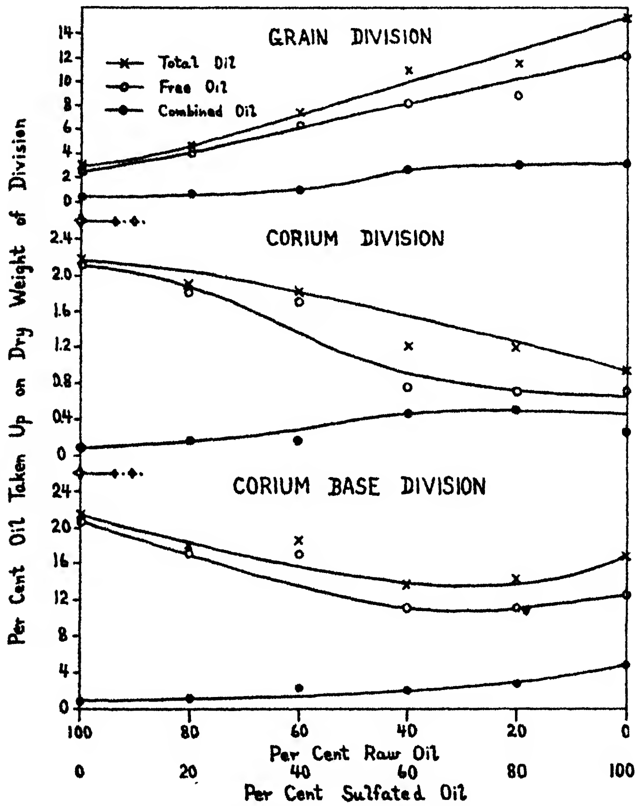


Figure 204. Effect of fatliquoring with sulfated cod oil-raw cod oil mixtures on the oil distribution in the horizontal divisions (Koppenhoefer-Retzsch).

chrome leather fatliquored with sulfated fatty oils. (3) Raw oil, the neutral oil fraction of sulfated oils, and the free fatty acids are all capable of lubrication of leather, and their efficiency appears to be in the order listed. (4) The difference in the distribution of neatsfoot, castor, and cod oil is capable of only partially explaining the character of the leather obtained.

Figure 204 shows the total, free, and combined oil, as found in the three

divisions of chrome leather when fatliquored with the indicated proportions of sulfated cod oil and raw cod oil mixtures, according to these investigators. Figure 205 is a comparison graph showing the tear and tensile strength of the whole leather obtained after fatliquoring with the same mixtures of sulfated cod and raw cod oil.

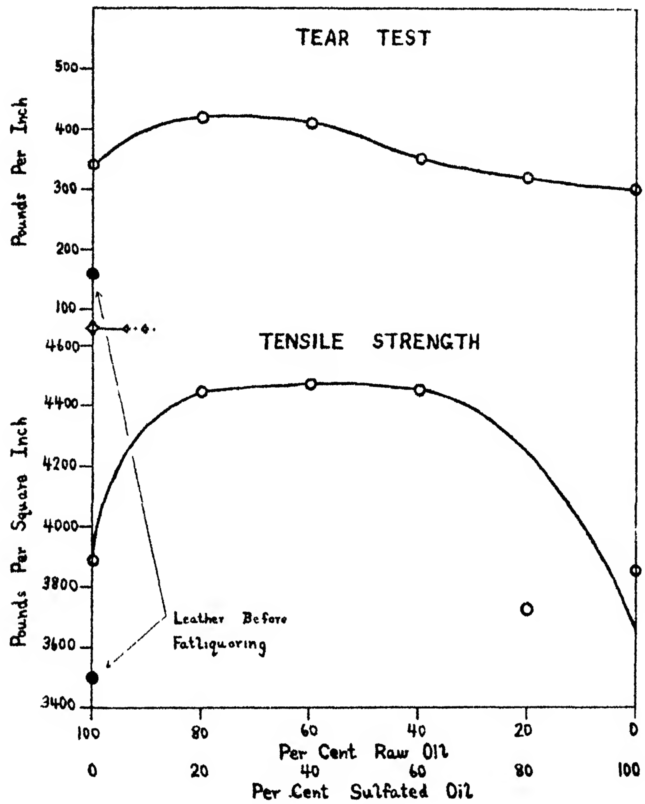


Figure 205. Effect of fatliquoring with sulfated cod oil-raw cod oil mixtures on the physical tests of calf leather (Koppenhoefer-Retzsch).

Effect of Oil and other Factors on some Physical Properties of Leather

It is well known that the kind and degree of tannage has a great influence on the shrinkage, stretch, and tensile strength of leather. However, it has also been found that the kind and amount of oil used has also a great bearing on the same physical properties of leather.

Nelles[41] has made a fine study of the effect that fatliquoring has on the

most important physical properties of leather. He found that good penetration of the oil gave increased suppleness and tensile strength, whereas a superficial penetration gave increased firmness. A shrinkage of 36 per cent in unoiled leather was gradually reduced with increased amounts of oil until it was 23 per cent when 10 per cent of oil was used. A comparison was made between sulfated neatsfoot and cod oils. Leather after neutralization was cut into similar samples, and treated with 4 per cent of each oil, at 70°, at pH values of 5.4, 6.9, 8.0, and 8.5 for neatsfoot, and 4.7, 6.0, 7.0, 8.0, and 8.5 for cod oil. The samples were dried at 30°-35° and finished.

They were tested for stretch and tensile strength after 24 hours, after storing for 15 days (25°), and after subsequent degreasing. The 24-hour tests showed that stretch increases with rising pH up to 8, and then decreases. Cod oil gave a little more stretch and a little less strength than neatsfoot, but this was not pronounced. After 15 days storage the strength and stretch increased slightly. These properties were not changed by degreasing, indicating that the effect was due to combined oil.

Stuffing and Oiling

These terms refer to the application of raw oils and greases (often high melting point greases and waxes) to leather, either wet or very dry, according to the process used. Some tanners refer to a fatliquoring procedure, in which emulsified solid fats and greases are used, as "stuffing." However, as used here, the term "stuffing" means the application of unchanged oils, fats and greases directly to leather. There are two general methods in common use: hand stuffing and drum stuffing of oils and greases applied to wet leather.

The application of oils and greases to wet leather is also commonly referred to as "currying." Balfe[4] and his co-workers have studied the most important factors that control the currying process and our knowledge in this field has been extended very considerably by their work. They showed that the excellence of curried leather depends on the extent of fine splitting of leather fibers which the currying oil or grease produces. The absorption of the currying mixture and the extent of fiber splitting depend on the viscosity of the oil and upon the interfacial tension existing between the currying oil and the wet leather fibers. They also showed formation of solvent-insoluble fatty material during storage.

Drum stuffing has the following advantages over hand stuffing: (1) greater speed; (2) uniformity of process both as to individual pieces and successive lots; (3) economy of greases; (4) heavier weights obtainable; (5) more waterproof leathers are possible.

The "Burning-in" and the "Hot-Dripping" processes are applied to very dry leather. In these procedures it is extremely important that the leather has been dried in a hot box for several hours; otherwise great injury may

result to the treated leather. The temperature commonly used in stuffing wet leather is about 50° (122° F), whereas in the "burning-in" or "hot-dipping" procedure temperatures upward of 90° (194° F) are used. The latter procedure has the advantages that much higher melting point fats and waxes can be used, that more fat can be incorporated in the leather, and that greater waterproofing properties can be imparted. The "burning-in" process involves placing the hot, dried leather on a table and pouring the hot, melted grease mixture on the flesh side only, thereby maintaining a good color on the grain. The "hot-dipping" procedure is accomplished by immersing the dried leather in a tank of the molten grease for about ten minutes. This gives a very dark colored leather.

The oiling or "oiling-off" procedure is quite commonly resorted to for many types of both light and heavy leather in the wet condition. In can be accomplished by either hand or machine and is frequently done after the leather has been fatliquored and horsed up overnight. In the case of sole leather, oiling is accomplished in a drum together with other materials, as will be described later. Rochow[49] made an excellent study of the oiling of leather and stated that the function of water in this procedure is to separate and swell the fibers. Oil can be absorbed by the smallest fibrils because it wets them in preference to water. The fatty oils and the corresponding fatty acids have more than twice as great a surface tension between the oil/air interface as between the oil/water interface. Mineral oils, however, show a surface tension about 1.47 times less between the oil/air interface than between the oil/water interface. This explains why fatty oils work so well when swabbed on wet leather and why the mineral oils when used alone give a stiffer leather.

Sole Leather Oiling and Finishing

The oiling of various type of upper leather and light leather is usually a very simple operation by itself; however, in the case of sole leather, the oiling is accomplished in conjunction with various filling, bleaching and anti-acid materials that are drummed into the leather together with the oils used. It is very seldom that a solid fat like tallow is used on sole leather, because it is very difficult to accomplish a clean take-up together with the heavy load of other materials. In determining the oil-wheel load for any given case it makes a great difference whether or not the procedure involves dry dipping. In the case of dry dipping, the sole leather is given approximately one-half the usual oil-wheel load the first time it arrives at that particular stage in the process. Then, after drying, it is taken back to the tan yard and immersed for about 50 minutes in the dry dip liquor made up of, for example, quebracho, syntan and spruce extract from 30° to 45° Barkometer (1.030-1.045 sp. gr.).

Out of the dry dip the stock is taken direct to the oil wheels for the second time. An illustration will be given of a sole leather oil wheel load as applied to a dry dip process. It is assumed that the stock has been bleached in the usual manner and wrung very well, then weighed and taken direct to the oil wheels.

For 1000 lbs of wrung sole leather backs the following load may be used as indicated before and after dry dipping.

	Tanner's Corn Sugar (lbs)	Epsom Salts (lbs)	Powdered Spruce Extract (lbs)	Boric Acid (lbs)	Sodium Acetate (lbs)	Mineral Oil (gal)	Sulfated Cod Oil (gal)	Raw Cod Oil (gal)	Wheeling Compound (lbs)
Before Dry Dip	28	14	8	1	0	1	1	0	0
After Dry Dip	30	16	10	3	2	1	½	½	10

The salts, powdered extract, boric acid and sodium acetate are added dry with the wrung leather and the drum run 10 minutes. Then the sugar is run in through the gudgeon at 150° F and the drum run 15 minutes. Finally, the oils and the compound are run in through the gudgeon in an emulsion made with an equal volume of water, and the drum run 10 minutes more. The stock is then taken to the drip tunnel for about 18 hours, then hung in the drying tunnels where it is dried slowly with a large volume of air, as cool as possible, but ending up at the dry end at a temperature of about 110° to 115° F. The time in the tunnels may take from 4 to 7 days.

The crust stock is dipped in a 20° Bk. sour dip made up with 2 parts sugar and 1 part epsom salts and then sammied overnight. The stock is then sponged by swabbing at 140° F and rolled once, piled down and re-rolled, then dried. After this it is washed on grain only by pouring or pumping at 140° F. The sponging or finishing solution and the wash solution as referred to above are made up as follows:

Sponging Mixture

20 lbs of Sponging Compound
10 qts. of emulsified mineral oil
(If kerosene is to be added, it should be mixed with the emulsified mineral oil.) The above boiled in about ¼ bbl of water, then made up to 50 gals and used at about 130°-140° F

Wash or Pouring Mix

10 lbs of Sponging Compound
1½ qts of emulsified carnauba wax
boiled in ¼ bbl of water. Tallow, borax, sulfated oils, etc. may be used. Then make up to 50 gals and use at about 140° F

A typical composition of a "sponging compound" as referred to above is as follows:

6.00%	Wool grease
2.00%	Tallow fatty acids
2.00%	Paraffin wax
1.50%	No. 1 yellow carnauba wax
7.00%	Rosin
2.00%	Glycerin
5.00%	30° Bé sodium hydroxide
6.50%	Coconut fatty acids
2.00%	Borax
4.00%	Gelatin
0.40%	β-Napthol
1.00%	Sodium benzoate
60.60%	Water
100.00%	

Oxidation of Oils

The subject of oxidation of oils is very important to the leather manufacturer in considering and choosing the best oils for his particular needs. It is well known in the leather industry that oils are more susceptible to oxidation on chrome-tanned than on vegetable-tanned leather, and it is generally thought that this is due to the catalytic action of the chromium salts. However, it is the writer's experience that the reduced chrome salts as found in leather are weak oxidation catalysts as compared to iron, cobalt, manganese, copper, etc On the other hand the vegetable tannins function as moderately effective anti-oxidants. A study[40] has been made of a number of marine and vegetable oils and their relative oxidizability determined; it was shown that the free fatty acids oxidized more readily than the corresponding neutral oils. A study[15] was also made of the susceptibility of oils to oxidation on various tanned hide powders; it was shown that oils oxidize most readily on chrome tan, but that the vegetable tans offer considerable retardation, and that the pyrogallol tans retard oxidation more than the catechol tans.

Fatty Spues and Resinification

The term "spue" is used to designate any exudation of any material that was once in the leather and is later expressed to the surface by either mechanical, physical, or chemical means.

Whereas in most cases spue consists of some form of fatty matter, nevertheless such exudations often may be of some other nature. Inorganic salts may spue badly, and free sulfur from one source or another, such as the use of hypo in chrome leather, may form a very aggravated case of spue that spoils the appearance and finish of the leather and is very difficult to remove. High melting point waxes and hard soaps may cause spue.

Fatty spues are of general occurrence and can come from either natural fats or added fatty matter. Moore[39] has studied spue on glazed kid leather and reported that this powdery type spue arises from lipid materials present

in the skins as received by the tanner, and that the most promising method of spue prevention, at present, seems to be the extraction of this lipid from the dry cured skins with a solvent. Many investigators have found that natural fat is frequently responsible for spue, and the reviewer knows of cases where the added fat made it possible for the natural fat to exude to the surface. Spues occur when the leather contains a sufficient amount of fats, loosely bound or in the free state, which can give either solid or resinous compounds. Almost all kinds of leather may show fatty spues under the many and various conditions necessary to bring this about. Natural fat often fulfills the conditions necessary for spueing, since it is often large in amount and is always loosely held and capable of giving solid products.

The composition of fatty spues has been found to consist largely or, in some cases, entirely of free fatty acids. This might make it appear desirable to use materials entirely free from fatty acids. However, this is usually not possible. On the other hand, Innes[23,24] reports that analyses of spuey and non-spuey leathers indicate that the tendency to spue formation increases both with amount of free fatty acids present and their melting points. The spuey leathers examined contained over 4 per cent free fatty acids with a melting point above 25°.

Stather and Sluyter[69] investigated resinous spues in vegetable leathers and found that these contained more free fatty acids than the fish oil used. Koppenhoefer[27] showed the analysis of powdery spue collected from glazed kid skin leather to be as follows:

Melting point (° C)	48.0
Acid value	116.6
Saponification number	205.8
Unsaponifiable	2.3
Cholesterol	0.0
Free fatty acid (calculated)	56.0

The same author found that solid acids represented 80 per cent of the total acids and that the epidermal lipids are not involved in powdery spue formation.

Balfe and Uryash[4] have investigated the resinous spues on vegetable-tanned leathers which had been curried with cod oil and found that these exudations are formed from the triglycerides and not from the fatty acids of the oil, since the free fatty acids in the spues and gums are formed by the oxidation at the double bonds of unsaturated radicals and not by hydrolysis of triglycerides. This is shown by the increases in the saponification values above the normal range of 185-195 for cod oil. They drew the conclusion from these investigations that the gummy spue on vegetable-tanned leathers is a polymerized oxidation product of cod oil. It is generally well understood in the trade that powdery spues are not caused by cod oil. Balfe[3] found that the removal of water-solubles from vegetable-tanned leather favored spue

formation, and that such spue occurred more readily on leathers tanned with catechol tannins than on the leathers tanned with pyrogallol tannins. Since this type of spue is caused by oxidation, Balfe's findings are in agreement with the reviewer's[45] work on oxidation, in which it was found that pyrogallol tannins retard oxidation of oils more than the catechol tannins. Balfe suggests the addition of pyrogallol tannins as an antioxidant; however, the reviewer believed that there are many commerical antioxidants available that are more effective than any natural tannins.

Balfe[3] also investigated the effect of free fatty acids in the oils used. A series of twelve cod oils, of free fatty acid contents ranging from 6 to 22 per cent, were impregnated into vegetable-tanned leathers which had been thoroughly extracted in water and dried before dipping in the oil. The results showed that the oils with low free fatty acid content tend to spue more rapidly than those of higher acid content. The more saturated the free fatty acids the more effective they are in preventing spue. This, of course, refers to the gummy resinous spue, not the powdery spue.

The mechanism of exudation or the reason why some fatty matter is expressed from the interior of the leather to the surface has been the subject of much conjecture and some study. The above investigators[4] have reasoned that the exudation of gummy spue is due to the fact that the polymerization which occurs during the oxidation of the cod oil gives rise to a lattice-like or three-dimensional molecular structure, which can neither form nor exist in the presence of the three-dimensional fibrous structure of the leather. Hence, if the tendency to form this lattice is sufficiently great, the oxidized oil must go to the surface, since this is the only place where the lattice can form freely.

Formation of Resinous Spues

Balfe[3] gives the following analytical results of vegetable-tanned leather, and a discussion of the cause of spueing or of its absence in each case.

Table 327

Sample No	Type of Leather	Grease in leather (%)	Water-solubles in degreased leather (%)	Free fatty acids in grease (%)
1	Belting leather, spued	17.7	5.3	9.6
2	Belting leather, not spued	14.2	13 9	62.8
3	Belting leather, not spued	15.0	7.1	54.0
4	Belting leather, spued	15.9	6 7	56.0
5	Belting leather, spued	17.4	8.8	24.8
6	Strap leather, spued	25.6	4.5	46.9
7	Rough dried vegetable tan heavy leather, spued	3.8	2.0	13.4
8a	Curried vegetable tanned upper, spued	18.9	7.6	40.4
8b	As 8a free from spue	19.5	9.2	47.9
9a	Black harness leather, spued	37 3	4 5	.
9b	As 9a free from spue	28.2	7.8	...
10a	Combination tannage, spued	34.2	6.2	13.2
10b	As 10a free from spue	32.8	10.5	13.4
11	Kip, spued	20.0	14.0	17.2

With the exception of numbers 7 and 11, all the leathers were curried with dubbin.

Sample No. 1. In this case the water-solubles and free fatty acid contents are insufficient to prevent spueing.

Sample No. 2. In this case the water-solubles and free fatty acid contents are sufficient to prevent spueing.

, Sample No. 3. Though the water-solubles content of this leather is low, it contains enough free fatty acids to prevent spueing.

Sample No. 4. The water-solubles and fatty acids in this leather should be sufficient to prevent spue; however, the sample had been exposed to sunlight, which presumably was the chief cause of spue.

Sample No. 5. The areas free from spue contained 0.1 per cent iron, whereas the spued areas contained 1.0 per cent iron, which is the main cause of spue in this case.

Sample No. 6. Spueing occurred on account of the low water-solubles and high grease content.

Sample No. 7. Spue occurred because of low water-solubles, notwithstanding low grease content.

Samples No. 8a and 8b. The differences in iron, water-solubles, and free fatty acids between the two leathers, though small, explain why one spued and the other did not.

Samples No. 9a and 9b. Sample No. 9a spued because it contained more iron and grease and less water-solubles than 9b.

Samples No. 10a and 10b. The greater amount of water-solubles in 10b has prevented spueing on this leather.

Sample No. 11. The grease extracted from this leather was of gummy consistency, had saponification value of 245, and contained 21.8 per cent of oxidized fatty acids. This indicates that the leather had been curried with cod oil only, and that the water-soluble and fatty acid content are not sufficient to prevent spueing of the large amount of cod oil in the leather.

In 1887 Eitner evolved a theory suggesting an explanation of the formation of resinous spues on leather. According to this theory, the finely divided unsaturated oil, on the fibers of the leather, absorbs oxygen, and the resulting oxidation and polymerization products are then expelled through the pores to the surface in the form of sticky drops by the action of heat and volume alteration.

Schulgin[62] has investigated many theories suggesting explanations of the formation of fatty crusts or solid powdery spues, and comes to the conclusion that the question as to why only fatty acids are expressed to the surface of the leather remains unanswered. He further reports that investigations on solid fatty spues by the Ukrainian Leather Institute have led to the following results: (a) The danger of solid fatty spues is not prevented even by

the use of fats of the best quality which are low in tallow content. (b) If leather is treated with solvents to remove fatty spues, no positive results are obtained. (c) The best and most lasting effect was obtained with birch tar oil. (d) It was found best to subsequently dry the spued leather. (e) The formation of solid fatty spues can be prevented if the leather is hot-dipped or stuffed by the "burning-in" process. In the last case, Schulgin suggests that the fat is in the form of a "fat-gel" impregnated into the fibrils, whereas, in the case of fatliquoring the fat is between the fibrils in the form of an "aquo-gel."

The natural hide or skin fat and its changes during the preparation of the leather has been studied by various investigators. Innes[24] has pointed out that the natural fat occurs predominantly in the fatty cells lying in the network under the grain layer. Thorough investigations by O'Flaherty and Roddy,[42] which make use of histological-microscopic methods, confirmed Innes' findings and also that part of the natural fat has emerged from the cells. Innes states that there is a splitting of the natural fat by enzymes inherent in the skin. Theis[73] observed, even during soaking, a splitting of the natural fat, while O'Flaherty and Roddy found no splitting in the soaks. Sharpening agents in the soak facilitate splitting, according to Moore and Highberger.[10] The above investigators have also studied the changes and removal of natural fats occurring in both the limes and the bate. Concerning the latter there is considerable contradiction; however, Schindler assigns to bating a large influence in the removal of natural fat, all of which has an important bearing on the tendency or freedom from spue of the eventual finished leather.

The fat-combining ability of (1) leather, and of the (2) hide-chrome complex, and of (3) the chrome salts, all have a bearing on the tendency of such leather to spue. Various investigators[51,55] have shown certain possibilities as (a) formation of compounds between fat and the hide or skin, (b) the entry of the fatty matter into the skin-chromium complex, (c) and the formation of chromium fatty acid compounds. There are also intermediate stages between solvent-resistant combinations and capillary absorption. Capillary forces can be strengthened to approach residual valency forces, and the possibilities of spueing are greater when there is merely capillary absorption than when there is firmer combination.

Distinction between spue on chrome leather and that on vegetable-tanned leather should be clearly outlined. Several references have been made in this section to the powdery or solid type of spue on chrome-tanned leather and the resinous or gummy type of spue on vegetable-tanned leather, and attention should be called to the different types of oils generally used on these two kinds of tannages. On chrome leather it is common practice to use neatsfoot oil and soap, or sulfated neatsfoot, either alone or with raw neatsfoot, or sulfated sperm oil or various vegetable oils, all of which can replace

the solid natural fat, which may come to the surface as a powdery spue. These same oils may in some cases be the primary cause of powdery spue, but not the resinous kind of spue which comes from the unsaturated fish oils so commonly used on vegetable leather. It is not common practice to use sulfated or raw fish oils on chrome leather unless such oils are blended with a substantial amount of mineral oil, which incidentally retards or prevents oxidation and spue. Schindler reasons that the capacity of chrome leather to bind fat in the capillary form is diminished by the entry of polar compounds into the complex; therefore highly sulfated oils may cause spueing leather.

A brief review is given of many factors, physical and chemical, that govern or influence formation of fatty exudations.

(1) Stather and Laufmann[68] found that ultraviolet light had a great effect on the oxidation of cod and shark liver oils, which manifested itself more by an increase in specific gravity and formation of oxyacids than by a drop in the Iodine Value.

(2) High heat (within the range of safety) has a tendency to fix the unsaturated fatty acids and thus retard spue. However, a fluctuating temperature, varying from above the melting point to well below the solidifying point of the solid fat, has a tendency to bring about formation of the powdery type of spue.

(3) The tendency to spue formation is increased with high relative humidity at a cool temperature, or especially if the leather is wet and dries in a cool atmosphere.

(4) Embossing or smooth plating at a hot temperature and high pressure, even though momentary, can melt and force to the surface solid fats or finishing waxes which will later crystallize and form a powdery spue.

(5) Various finishes such as seasonings and pigment finishes may cause spue if they contain hard waxes, especially if the leather is embossed.

(6) The free sulfur deposited on the fibers in the leather from the use of hypo in chrome tanning is more likely to crystallize on the surface if the leather is embossed.

(7) The liberation of moisture in the leather during smooth plating or embossing can cause spue.

(8) Insufficient neutralization of chrome leather can cause spue.

(9) The added fat can replace the solid natural fat or serve as the vehicle by means of which the solid fat can come to the surface.

The effective prevention of spue, which is a perpetual question in the tanning trade, can only be generalized on. It is self-evident from the discussion of this problem given above that the removal of as much natural fat as possible by various means, and the elimination of the many other factors

that contribute to the formation of spue after the leather is finished, will do much to alleviate this very annoying and often serious problem.

This chapter should properly end with a detailed correlation of the kind and amount of the oils and fats used and the resulting physical properties obtained in the finished leather. However, this is not possible as yet because there is not sufficient information available in the literature of leather to make such an undertaking feasible.

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Chapter 25

Physical Testing Methods

The American Leather Chemists Association and the leather committee of the Federal Specifications Executive Committee have been diligently at work during the past five years, developing and improving methods for measuring the physical properties of leather. It is not surprising, therefore, to find that the treatment of this subject in the second edition of this monograph has become inadequate for meeting present demands.

Much emphasis is now being placed on the need for this kind of information, in contradistinction to information about the purely chemical characteristics of leather, which was formerly thought to be the sole end of testing. This is set forth in detail in Part A of this Section.

The number of specimens to be tested was formerly a figure chosen empirically and haphazardly, such as, "Test three samples per carload" or "Two per cent of the number of pieces in the shipment." The application of statistical theory as developed within the past ten years now enables us to compute the number of specimens which must be tested if we are to have a specified degree of assurance of the correctness of the result. In Part B, it is shown that in general we should test not less than seven specimens, and need not test more than 20.

Part C tells how the effect of water in the leather can be accounted for. This effect does not vary in proportion to the amount of water, nor does water have the same effect on different properties. It is therefore necessary to "condition" leather before testing it, by bringing it into equilibrium with a standard atmosphere.

Part D gives verbatim transcripts of 17 methods which have been prepared by the two groups named above. In each case, the latest wording is given, whether the method has been officially adopted, whether it is provisional or tentative, or whether it has merely passed the first stage of committee acceptance.

Finally, attention is called to the fact that these methods will be subject to continual changes for years to come. The present methods will be altered, and new methods will be developed, as we benefit from the research work now under way.

A. Reasons for Making Physical Tests

Before describing the methods themselves, it is worth while to discuss the purpose of testing. What is to be accomplished by means of the tests, to justify the expense of making them?

Leather may be tested to find out whether or not it conforms to the description included in the purchase contract. Does it meet the requirements of the specification which is the basis on which the buyer and seller have agreed to do business?

It may be tested to provide data for purposes of design. If plans are being prepared for an article which contains an element of leather under tension, what must be the size of the leather piece to withstand the predetermined tension for the predetermined time?

Leather may be tested to determine its suitability for a given purpose, or to compare it with another piece of leather or with a similar piece of some competitive material.

The fact that testing costs money must never be lost sight of. The buyer of the leather always pays for the testing, whether he knows it or not. In every case, he must answer the question: Will the information obtained from the test be worth the cost of getting it?

The cost of testing must include such items as the charges for the capital investment in expensive laboratory equipment, the labor cost of trained personnel, the cost of the material destroyed during the testing, and the cost of issuing the report and perhaps servicing it by court testimony. It therefore follows that the methods should be designed to use ordinary equipment wherever possible, that the tests should be simple and rapid, and that the sample should be taken in such a way as to conserve the value of the piece sampled.

Assuming that the testing machines are kept carefully calibrated and in good working order, and that the testing is done with reasonable skill, the value of the information obtained will depend on the interpretation of the data and on the adequacy of sampling.

The leather industry has been built up on the basis of chemical tests. Tanning is a chemical engineering process, and tanneries are therefore equipped with excellent chemical laboratories, which are necessary for adequate plant control. Moreover, there are some experts in the industry who have sufficient skill and experience so that they can tell, from a study of the chemical composition of a piece of leather, just about what the leather is like. In the usage of leather, we are, however, interested mainly in the physical, and not the chemical characteristics.

In most cases there is no definite quantitative relationship between the chemical composition and the physical properties of leather. For example,

we have reason to believe that too much water-soluble material in sole leather will decrease its durability, but when tests are carried out to learn how much is "too much," the data are disconcertingly conflicting.

If we are interested in the durability of sole leather, why not measure it directly, rather than analyze the leather to determine its chemical composition and then try to interpret the results in terms of durability?

Physical tests are to be preferred to chemical tests in all such cases, where the customer is directly interested in the performance of the leather. True, the kinds and amounts of the chemical constituents may be the determining factors which cause the leather to have certain properties, but the customer is more interested in the properties than he is in what causes them.

Before deciding whether or not to have any testing done, the customer should have clearly in mind just what information he can get, and, more important, what information he cannot get, from the data.

While the physical properties of leather may in general be regarded as characteristics inherent in the material, nevertheless the measured values are importantly affected by the shape and size of the finished article and of the test specimen. The length of time during which the property is utilized will also affect its numerical value. For example, one may find that a certain lot of leather has a "tensile strength of 2,500 pounds per square inch." This information was obtained by breaking several pieces of leather, each $\frac{1}{2}$ inch wide by $\frac{1}{8}$ inch thick, at a temperature of 70° F, and having a moisture content in equilibrium with air at 65 per cent relative humidity, the load being applied at such a rate as to break the leather in less than a minute. One might conceivably extrapolate this information to mean that a piece of the same leather $\frac{1}{4}$ inch thick by 2 feet wide (6 sq in) could sustain a load of 15,000 pounds (6 × 2500), for a year exposed to the weather. Such an extrapolation is certainly unjustifiable and might lead to dangerous conclusions.

The result of any physical test must be considered with full information about the way in which the test was made. Experience is required to interpret the test data in terms of useful information.

But the greatest value of physical testing methods to the leather industry lies in their use as tools for research. They enable one to ascertain whether or not an experimental change in the tanning process causes a real improvement in the quality of the leather. A careful study of the effect of well-planned physical or chemical treatments on certain physical properties can bring to light facts about the structure of the leather and thereby increase our basic knowledge.

B. Sampling

The physical testing of leather usually involves the destruction of the pieces tested. Obviously one cannot test all the pieces delivered. It is

therefore necessary to select a certain few to be tested, so that the results will bear a sufficiently close relation to the results which would have been obtained if all had been tested. The care with which this selection is made is one of the factors which determines the value of the test data. If the sample does not adequately represent the lot, the test data will be of dubious value, and may well be misleading.

It is obvious from the above that we must have enough samples to represent the lot. Consideration of the cost of testing dictates that we shall test no more samples than necessary. The number to be tested is therefore quite critical. Statisticians have developed a way of computing this number.[1]

The method is based upon certain assumptions, which are probably not strictly correct for leather. But the final result is reasonable and practical, even if not entirely accurate. The method is expressed by the formula

$$n = \frac{T^2O^2}{E^2},$$

where

n = number of specimens to be tested
T = probability factor
O = standard deviation, in per cent
E = allowable random sampling error of the average in per cent

The standard deviation must be computed from a comparatively large number of tests of the material if it is to be accepted as reliable. It is the square root of the average of the squares of the deviations of the test results from their average. Both the property and the material must be specified: one may speak of the standard deviation of the tensile strength of sole leather; one would expect the standard deviation to be different for the stretch of sole leather, or for the tensile strength of light leather. The standard deviation will also change with location in the hide with different tannages, and with similar factors, which will be considered later. For the present, it must be assumed that enough tests have been made to establish the standard deviation as a fixed property of the leather under consideration.

The values of E and T have nothing to do with test data; they must be selected by the operator on the basis of his judgement and of the economics of the case. For much leather testing it has been agreed that results will be satisfactory if the changes are 9 to 1 (probability factor for 9 to 1, $T = 1.645$), that the average value found from testing the samples is within ± 10 per cent (allowable error, $E = 10$) of that which would have been found if all the leather had been tested. These values of T and E can therefore be inserted in the above formula, if one is satisfied with these conditions.

A study of the formula reveals the fact that if we want an assurance of 99 per cent ($T = 2.58$) rather than the 90 per cent used above, we must test

$\frac{2.58^2}{1.645^2}$, or nearly $2\frac{1}{2}$ times as many samples. If the result is to be within 5 per cent rather than the 10 per cent assumed above, proportionately more samples must be tested.

It is well known that the physical properties of leather vary with the location in the hide. The flabby portion of the belly may differ from the firm portion over the kidneys by 50 per cent or more of the average for the whole hide. Different hides will have different properties, even when tanned by the same process, and the use of different tanning processes brings in another variable.

When sampling for chemical analysis, it is customary to cut samples from three locations in each hide, so chosen that the average composition of the three will closely approximate the composition of the whole hide. This logic seems faulty when an attempt is made to apply it to physical testing. There would seem to be no reason why anyone would be interested in the average tensile strength of a whole hide. Leather is not used that way. Rather, it is necessary to know how the tensile strength varies throughout the hide, and select for use that portion which has the desired strength. Testing is then conducted to show whether or not this hide is at least as strong as desired. For this purpose, it is more logical to take one specimen from a predetermined location in each hide, and assume that the percentage variation will be about the same throughout each hide. In testing a car-load of leather belts, it is obvious that more information will be obtained by testing one sample from each of ten belts rather than ten samples from one belt.

Beek and Hobbs[2] had at their disposal many years' accumulation of test data on the tensile strength of heavy leather. They were therefore able to make an intensive study of the variations in the standard deviation within a bend. When the average value was inserted in the equation given above, with due regard to engineering judgment in the use of the equation, they developed three conclusions which have been generally accepted by the Federal Specifications Committee.

(1) Not more than one test specimen should be taken from any piece of leather. The reason for this has just been explained.

(2) No matter how small the lot of leather, at least 7 pieces should be sampled and tested. If there are not seven pieces in the lot, or if the cost of testing is too great in comparison with the cost of the lot, it is better to waive the testing.

(3) The additional information which can be obtained by testing more than 20 samples from a single lot is so small as to be not worth while. Therefore 20 samples may be taken as a maximum, regardless of the size of the lot being sampled. The above reasoning has led to the development and tentative approval of the following method.

Federal Specifications Executive Committee Sampling Leather for Physical Tests[a]
(Proposed 1943)

Number of pieces in sample. From any shipment of leather products, the number of pieces shown in Table 328 shall be taken, so selected that they will be fairly representative of the shipment.

Table 328

No pieces in shipment	No. pieces to be taken as samples
100 or less	7
101 to 250	9
251 to 500	10
501 to 1000	12
1001 to 5000	16
5001 to 10,000	17
over 10,000	20

Note: The average of the test results from specimens cut from fewer than 7 pieces cannot be expected to give a fair picture of the quality of the lot. The testing of specimens from more than 20 pieces does not give sufficient additional information to be worth while. Each test specimen should be cut from a different piece. When, in the judgment of the purchaser, the size of the shipment is too small to justify the testing of seven specimens, the testing may be waived.

Location and size of specimens. Leather samples for use in making physical tests shall be cut as directed below: (see Figure 206).

Determine the amount of material needed by adding together the requirements for each test to be run.

(1) For *skins, hides, sides, crops* and *backs:* cut the test specimen approximately square and as large as needed with one edge parallel to and 1″ in from the backbone line. Cut from tail to head beginning at a point distant from the root of the tail one-sixth of the length of the piece (A).

(2) For *bends:* cut the test specimen approximately square and as large as needed with one edge parallel to and 1″ in from the backbone line. Cut from tail to head beginning at a point distant from the root of the tail one-fourth the length of the piece (B).

(3) For *belting butts:* and *belting butt bends:* cut a strip 2″ wide the full length of the butt or bend, so located that its long-dimension center line is parallel to the backbone and half way ± 1″ (C) between the backbone line, and the belly edge. Cut specimens for physical tests from this strip at a location beginning one-fifth the distance from tail to shoulder (D) and extending forward as required to obtain the desired number of test pieces.

(4) For *bellies:* cut the test specimen 2″ wide and as long as needed with one long-dimension edge parallel to and 1″ in from the belly line and in such manner that one end falls on the line dividing the "hind shank" from the "belly center."

(5) For *double shoulders:* cut a test specimen 2″ by as long as required, with one long-dimension edge parallel to and on the cut edge and located in such manner that its center is equidistant from the back bone and the belly edge.

C. Conditioning

The physical properties of leather are directly related to the amount of water which it contains, which is normally of the order of 10 per cent. This amount is not constant; it is continuously varying with the changes of relative humidity in the air surrounding the leather. The water in the leather constantly exerts a pressure, trying to get out; and the water in the air constantly exerts a pressure, trying to get in. When these pressures are equal, water goes out of the leather at the same rate that it goes in and the system is said to be in equilibrium.

Unless artificially controlled, the relative humidity of the air varies

[a] Credit for this method is due largely to J. S. Rogers, U. S. Dept. of Agriculture.

continuously. It may approach 10 per cent in a heated room in the winter or go to about 90 per cent in the same room on a rainy day in summer. Leather in equilibrium with the air may thus contain anywhere from 7 to 30 per cent of water, and its tensile strength may vary from 4900 to 5200 pounds per square inch, solely because of this variation in the water content.[6]

For ordinary routine testing, it may well be that this variation is not of sufficient importance to justify the installation of expensive equipment to bring the humidity under control. But for research work, or for referee test-

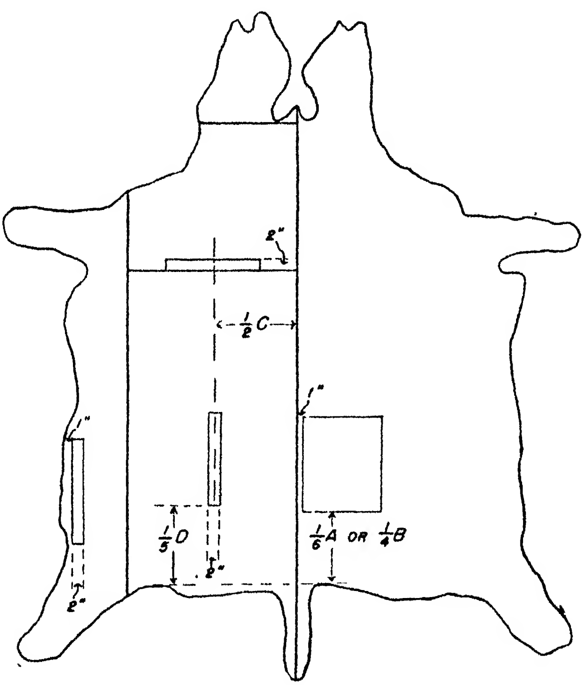


Figure 206. Locations of specimens.

ing to settle disputes, the matter may not be overlooked; it is necessary that the leather be brought to some predetermined standard of moisture content before it is tested.

In air at 90 per cent relative humidity, a piece of chrome-tanned leather may contain 38 per cent of water, while a piece of vegetable-tanned leather right beside it may contain only 25 per cent. To adopt a rule that the leather shall be conditioned until it contains x per cent of water might require an atmosphere of different relative humidity for each sample. It would certainly require previous analysis of the leather, computation of the weight the specimen would have when it contained the x per cent of water, and many

and frequent weighings to find out just when the correct weight was reached. A more important argument, however, is that different leathers are not brought to the same water content when used, and therefore should not be when tested.

To overcome this objection, and to make the conditioning method practicable, it has been generally agreed to bring the leather into equilibrium with a standard atmosphere, and let the water content be what it will.

This method is still not entirely practicable, for to bring the leather into equilibrium, if taken literally, may require a very long time. The rate at which leather will take up water from the air will depend partly upon the nature of the leather and how hard it has been rolled; partly on its chemical composition and how much grease it contains. The rate of attaining equilibrium is also dependent upon its thickness. The effect of the above factors, and others not enumerated, is that the water content of the leather will approach asymptotically that required for true equilibrium.

The practical answer to the above problem is to specify that the leather shall be considered to be in equilibrium with the standard atmosphere when its weight remains practically constant (within ± 0.1 per cent) for a reasonable length of time (one hour).

One must remember the "hysteresis effect," however. The leather will contain more water when brought into equilibrium from the overwet condition than it will when it is too dry to start with.

There remains only, therefore, selection of the standard atmosphere. Many arguments are presented as to why one set of conditions is better than another set, but the fact remains that there is no theory which can be brought forth to support the selection of any particular conditions, within wide limits. Some people prefer high humidity because, when tested in such an atmosphere, the leather will have higher strength, and they want the results to be impressive. Others prefer low humidity, claiming that the operating cost of the conditioning equipment is less. Still others want to test leather in the same atmosphere as that now required for some other material, such as textiles, so that the same conditioning room can be used for both. This last group is the dominant factor at present; the standard atmosphere for testing leather is fixed at 65 per cent relative humidity and 70° F because this is the atmosphere which has been used by the textile industry for many years, and many commercial and governmental laboratories which are equipped to test textiles are now called upon to test leather. It must be emphasized, however, that the conditions selected have no basis other than an agreement within the industry.

Laboratories not equipped with a room with humidity control may condition the leather in a desiccator over a saturated solution of cobalt chloride if a few precautions are observed. The salt solution must be saturated, as

shown by the presence of undissolved salt, and the temperature must be closely controlled. In order that the water content of the leather shall not change during the process of transferring the specimen from the desiccator to the testing machine, this transfer should be made as quickly as possible. and the atmosphere surrounding the testing machine should not be too different from the standard atmosphere.

A.L.C.A. Method of Conditioning Leather for Physical Tests
(Adopted as Official, 1941)

Samples shall be conditioned prior to testing in an atmosphere of 65 per cent ± 2 per cent relative humidity and at a temperature of 70° ± 5° F until they have reached equilibrium, and shall be tested in that atmosphere. Samples shall be considered as having reached equilibrium when weighings made at hourly intervals show no progressive change in weight and no change greater than ± 0.1 per cent. In general, at least 24 hours will be required to reach equilibrium.

If a conditioning room is not available samples may be conditioned in a desiccator or suitable chamber and removed therefrom one at a time and tested, provided: That the test is completed within 10 minutes and that the atmosphere in the room in which the test is made is not less than 50 nor more than 65 per cent relative humidity and not less than 65° nor more than 75° F.

The required relative humidity in the desiccator or chamber may be maintained with a saturated solution of cobalt chloride or a 36 per cent solution of sulfuric acid, the former being preferable.

D. Methods of Testing

Area and Thickness. A hide is quite irregular in outline and to measure its area with a rule would be extremely laborious. Commercially, such areas are measured with an integrating device. The hide is passed over one large roller and under a battery of smaller rollers. The smaller rollers register when raised by the hide. The machine notes how long each roller has been raised from its zero position, adds the data together, and gives the answer in square feet. It is necessary to calibrate these machines and for this purpose (and for referee tests) a planimeter is recommended.

In measuring the thickness of leather the precautions which apply to all easily compressible materials must be observed.[5] Most devices for measuring thickness involve applying a load to the specimen, or pinching it between two jaws. The pressure makes the leather thinner. Reproducible results can be obtained, therefore, only when this pressure is carefully controlled. Since the "thickness" is thus seen to be a function of the pressure used in measuring it, everyone must agree to use the same pressure, else the thickness will be quite indefinite.

There are two devices in general use for measuring the thickness of leather. One consists of a pair of jaws connected through a hinge and held together by a spring. The specimen is pinched between the jaws, under the pressure exerted by the spring. This device is commonly used in tanneries. It enabled one to measure the thickness of the hide six inches or more in from the edge. The other device is a dead-weight micrometer, for measuring the

thickness of test specimens in the laboratory. This consists of a horizontal anvil on which the specimen is laid. The presser foot is lowered onto the specimen, and exerts a predetermined pressure.

In both devices, the readings are magnified. In the spring gage, the smallest scale division is one-half of one sixty-fourth of an inch; in the micrometer, it is one-thousandth of an inch.

The leather industry has inherited a peculiar jargon: the thickness of heavy leather is usually expressed in irons, one iron being one forty-eighth of an inch. The unit of thickness of light leather is the ounce, equal to one sixty-fourth of an inch. In the laboratory, however, test specimens are measured in thousands of an inch.

Because each graduation on the spring gage is one-half of one sixty-fourth of an inch, tanners have come to speak of the thickness of leather to the nearest half-ounce, and figures of this order are found in specifications. One should remember that the thickness of a piece of leather is dependent on the pressure used in measuring it, and that neither surface of the piece is smooth and plane. To specify thickness to $\frac{1}{128}$th of an inch is manifestly absurd.

The moisture content of the leather has a significant effect upon its dimensions. The leather must be conditioned when measured.

A.L.C.A. Methods for Measuring the Area and Thickness of Leather
(Official--1941)

Width and length of test specimen: Width and length shall be measured with a steel scale graduated to fiftieths of an inch, and the dimension shall be measured to the nearest half division.

Thickness of test specimen: Thickness shall be measured with a dead-weight type of gage equipped with a dial graduated to read directly to one-thousandth of an inch The presser foot shall be circular with a diameter of three-eights of an inch ($\pm$ one thousandth inch). The presser foot and moving parts connected therewith shall be weighed so as to apply a total load of 200 grams $\pm$ 2 grams to the specimen. Thickness shall be measured to the nearest thousandth of an inch.

Thickness of commercial units: For measuring the thickness of commercial units, but not of test specimens, a spring actuated gage shall be used. This shall be graduated to read directly to one-half of one sixty-fourth of an inch The contact foot shall be five-sixteenths of an inch in diameter. The spring shall be so adjusted that it will exert a total load on the foot of one pound when the gage reads one thirty-second of an inch, and two pounds when the gage reads three-sixteenths of an inch.

Area: For the precise measurement of the area of a large piece of irregular shape, or for the calibration of a commercial leather measuring machine, a planimeter shall be used. This shall have a tracer arm approximately forty inches long. The measuring wheel shall be graduated to read directly to one-fourth of a square foot, and spaces between graduations being about one-tenth inch. The measuring wheel shall have a capacity of ten square feet, and the planimeter shall be equipped with an auxiliary wheel so that the total capacity of the instrument is at least forty square feet. Measurements shall be made to the nearest twentieth of a square foot. A vernier scale may be attached to the planimeter to facilitate reading.

Tensile Strength. The tensile strength of leather is of obvious importance in connection with such uses as transmission belting, harness, etc. In other cases, where its importance is less obvious, it is nevertheless a valuable criterion for judging the quality of the leather.

While tensile strength should probably be considered to be an inherent property of the material, like specific gravity, we must not lose sight of the fact that the figure for tensile strength as determined by testing is dependent to a large extent on the various factors which must necessarily accompany the making of the test.

The quantity of water which the leather contains at the time it is tested has, as indicated above, an important effect on the strength. Tensile specimens must be conditioned.

The shape and size of the specimen will determine the way in which the stress is distributed and will therefore affect the test results. There are three kinds of specimens in general use.

Heavy leathers are cut into dumb bell-shaped specimens, as shown in Figure 207. A steel die must be used for cutting these specimens, and the cutting edges of the die must be straight on the inside and tapered on the

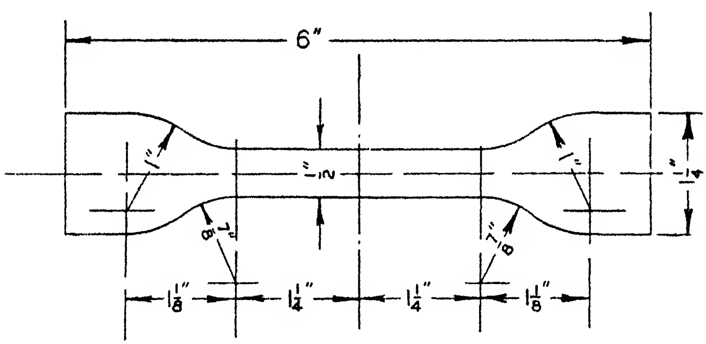


Figure 207. Shape of specimen for testing strength and stretch.

outside; otherwise there may be a difference of 10 per cent or more in the width of the two sides of the specimen. The peculiar shape was intended to concentrate the stresses in the narrow portion of the specimen. This ideal has not been realized, so that it is imperative that the dimensions and shape indicated in the figure be followed exactly.

The grab method is used in testing light leathers, including shoe upper leather. The specimen for this method is a rectangle, 3 by 6 inches. The jaws of the testing machine are so arranged that the direct pull comes on an area one inch wide and three inches long, in the center of the specimen. Because of the natural distribution of stresses, some of the load is taken up by the part of the specimen which is outside the jaws, and the actual width under strain is not known. Also, light leathers like suede, chamois, etc., have rough surfaces so that their thicknesses can not be precisely measured. It is therefore customary to make no attempt to ascertain the cross-sectional

area of the specimen, but to report the test result in pounds rather than in pounds per square inch used in reporting results obtained by testing dumbell specimen.

The third method is used in testing those kinds of leather articles which come to market in forms too narrow to permit cutting dumbbell specimens. These include laces, welting, straps, etc.

The actual testing is conducted with a motor-driven testing machine, like that shown in Figure 208. The capacity of the machine has an effect on the results. A big, heavy machine is not likely to give a high degree of precision when used to test a light, weak piece of leather, and the opposite extremes will be equally unsatisfactory.

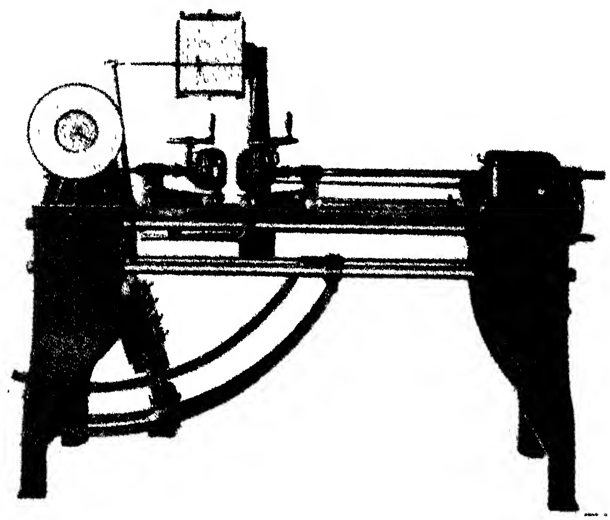


Figure 208. Machine for measuring the tensile strength of leather.

The effect of the speed of the machine has been the subject of a bit of research.[7] When the strength is plotted against the speed, the curve has a double inflection. The strength is practically constant at speeds from 6 to 12 inches per minute; speeds below 6 may give an apparent increase of the order of 4 per cent in the strength; speeds above 12 will give a corresponding decrease.

A.L.C.A. Method for Measuring the Tensile Strength of Leather

(Adopted as Provisional, 1941. Amended 1942)

Apparatus: The testing machine shall be motor-driven. The speed of the moving head when running free shall be 9 ± 1 inches per minute. The capacity of the machine shall be such that any readings taken during or at the completion of a test will fall within

the loading range, which is defined as that within which the indicated load has been shown by calibration to be correct within ± 1 per cent.

Samples: A sample to represent a shipment shall consist of seven specimens, each taken from a different piece of leather. The location from which each specimen is cut shall be as specified by the American Leather Chemists Association

Kinds of Specimens: (a) For testing belting, hydraulic, rigging, strap, sole, and harness leather, the specimen shall be cut with a steel die the sides of which are straight on the inside and tapered on the outside. The specimen shall have the following shape and dimensions. (b) For leather products 0.5 inch or less in width, such as welting, straps, and laces, the specimen shall be 6 inches long. (c) For boarded, buffed, or embossed leathers, such as chamois, suede, bag, case, and upholstery, the thickness of which cannot be accurately measured, the specimen shall be 3 x 6 inches.

Conditioning: The specimen shall be brought to equilibrium with an atmosphere of 65 ± 2 per cent relative humidity and 70° ± 5° F, and tested in that atmosphere (see Am. Leather Chem. Assoc. method).

Procedure: (a) Use specimen described in subsection "a" above Measure (see ALCA method) the thickness at five places along the restricted portion. Make two measurements of the width, both taken at the center line, one on the grain side and one on the flesh side. Compute the cross-sectional area from the average of the thicknesses and width measurements, to the nearest thousandth of a square inch. The jaws of the machine shall be not less than 1 5 inches wide, and 4 inches apart at the beginning of the test. (b) Use the specimen described in subsection "b" above. The jaws of the machine shall be not less than 0.25 inch wider than the specimen, and 3 inches apart at the beginning of the test (c) Use the specimen described in subsection "c" above. One jaw of each pair shall be 1 inch wide; the other, not less than 2 inches wide The jaws shall be 3 inches apart at the beginning of the test.

Report: The report shall show which of the three methods (a, b, or c) was used It shall show the average, maximum and minimum breaking loads for the 7 specimens. If method (a) was used, these shall be computed to pounds per square inch. If methods (b) or (c) was used, the breaking load shall be reported in pounds.

Elongation

The percentage elongation of a piece of leather under an applied load is an indication of the quality, and is also a direct measure of one of those properties which make leather peculiarly adapted to certain uses. If the leather stretches more than it should, it is probably weak; if less than it should, the leather has probably begun to harden and turn brittle. The consumer avoids the acceptance of such leather by placing a minimum requirement on tensile strength and a minimum requirement on elongation.

Since elongation is measured simultaneously with tensile strength, and on the same specimen, the conditioning of the sample, and the machine variables, are governed by the method for measuring tensile strength.

The method of measuring elongation is not satisfactory: it is difficult and is not precise. The operator is supposed to keep one eye on the dial of the testing machine to see when the predetermined load is reached. With the other eye and both hands, he must operate a pair of dividers to follow the gauge marks on the leather as they separate. The dial and the gauge marks may be in such relative positions that two operators are required. The separation of the gauge marks is supposed to be measured to the nearest fiftieth of an inch, which is one per cent of the original two inch length. It is not likely that the measurement will be made with this degree of precision.

A.L.C.A. Method for Measuring the Elongation of Leather
(Proposed 1942)

Specimen and Apparatus: To measure the elongation, the same types of specimen and of equipment shall be used as are called for in the Method for Measuring Tensile Strength.

Gauge mark: When a dumbbell shaped specimen is used, two parallel gauge marks, 2 ± .01″ apart, shall be stamped on the specimen, equidistant from the center line. They shall be stamped in such a way as not to injure the leather.

When the specimen is other than dumbbell shaped, the distance between the clamps shall be used instead of gauge marks, and this distance shall be 3 ± .01″ at the beginning of the test.

Conditioning: The specimens shall be brought to equilibrium with an atmosphere of 65 ± 2 per cent relative humidity and 70° ± 5° F, and tested in that atmosphere.

Procedure: While the testing machine is running, follow the distance between the gauge marks or the clamps with a pair of dividers. The test is completed when the load reaches a predetermined value or when the leather breaks, whichever is called for by the specification. Measure the distance between the divider points, using a steel scale graduated to fiftieths of an inch.

Report: Compute the increase in the gauge length as a percentage of the original gauge length, and report as per cent stretch at the specified stress.

Tearing Strength

It has been found necessary to have five different methods for measuring the tearing strength of leather. The "tongue" tear is designed to measure the ability of leather to resist tearing as the term is generally understood. The "split" tear is the same as the "tongue" tear, except that the force is applied in such a way as to split the leather—to tear the grain surface from the flesh surface. The "buckle" tear is designed to measure the strength of the joint made by a buckle. There are two "stitch" tears, one for heavy leather and one for light, both designed to measure the ability of the leather to hold stitches.

The operator must select the method applicable to the particular case, and must state in his report which method was used.

In both of the stitch tear tests, wire is used instead of thread. It has been found that thread stretches during the test, and its diameter decreases. The magnitude of this decrease depends partly on the quality of the thread and partly on the strength of the leather. A thread of small diameter will tend to cut through the leather and give a low reading. It is therefore better to use a wire of sufficient strength so that the reduction of diameter during the test will be negligible.

F.S.E.C. Methods for Measuring the Tearing Strength of Leather
(Proposed 1942)

When measuring the tearing strength of leather by any of the following methods, the specimen shall be prepared in such a way that the tear is made in a direction parallel to the backbone, unless such direction can not be ascertained from a visual examination of the sample.

Method A. Tongue tear. Prepare a specimen 6 inches long by 1 inch wide. Punch a hole $\frac{3}{16}$ inch diameter on the center line of the specimen, the center of the hole being 1 inch from one end. Slit the specimen along the center line from the hole to the further end, forming two tongues. Clamp these tongues in the jaws of a tensile testing machine, the distance from each pair of jaws to the common base of the tongues being three inches. The

jaws shall be not less than $\frac{3}{4}$ inch wide. Report the maximum pounds tension required to tear the leather.

Method B. Split tear. Prepare a specimen five inches long by one inch wide. Split it for a distance of three and one-half inches from one end by cutting as nearly as practicable along the middle line of the thickness, forming two tongues. Clamp these tongues in the jaws of a tensile testing machine, the distance from each pair of jaws to the common base of the tongues being two inches. The jaws shall be not less than one and one-fourth inches wide. Report the maximum pounds tension required to tear the leather.

Method C. Buckle tear. Prepare a specimen eight inches long and one inch wide. Punch a hole three-sixteenths inch diameter on the center line of the specimen, the center of the hole being three inches from one end. Prepare a one and one-fourth inch harness buckle with a tongue seventeen hundredths to eighteen hundredths of an inch in diameter, secured to a strap. The strap shall be supplied with a keeper, and the distance from the base of the tongue to the end of the strap shall be five inches. The shorter end of the specimen shall be passed through the buckle, the tongue of the buckle fitted into a hole in the specimen, and the end of the specimen passed under the keeper. The longer end of the specimen shall be gripped in the other pair of jaws of the testing machine, the distance from the jaws to the hole being three inches. The jaws shall be not less than one and one-fourth inches wide. Report the maximum pounds tension required to pull the specimen out of the buckle.

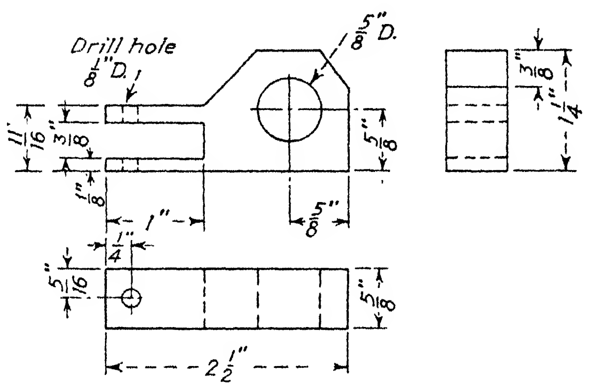


Figure 209. Block used in measuring tearing strength.

Method D. Stitch tear. Prepare a specimen two inches long by one inch wide. Drill a hole one-eighth inch in diameter, the center of the hole being on the longitudinal center line of the specimen, five thirty-seconds of an inch from one end. The end of the specimen further from the hole is clamped in one pair of jaws of the testing machine. The other pair of jaws is removed from the head and replaced by a block shown in Figure 209. The specimen is attached to the block by passing a piece of No. 13 Birmingham gage iron wire through the holes in both. The jaws shall be not less than one and one-fourth inches wide, and the distance from the jaws to the near edge of the block shall be one inch at the start of the test. Report the maximum tension required to pull the wire through the leather, and also the minimum thickness of the specimen in the region surrounding the hole.

Method E. Stitch tear for light leather. Prepare a specimen two inches long by one inch wide. Punch two holes in the specimen with a punch having an outside diameter of $\frac{5}{32}$ inch. The holes shall be $\frac{1}{4}$ inch apart, $\frac{3}{4}$ inch from the shorter edge of the specimen and centered on the longer center line of the specimen A soft wire, 0.04" ± 0.005" in diameter shall be threaded through the holes in such a way that both ends project from one side of the specimen. These shall be placed in one jaw of a testing machine and the specimen in the other jaw. The report shall state: (1) the number of pounds required to pull the wire through the leather; (2) the thickness of the leather; (3) whether the wire was pulled through from the grain side or from the flesh side.

Bursting Strength

The measurement of bursting strength has been found to be of great practical convenience to the paper industry, the strength of paper being usually referred to in terms of "points, Mullen." Perhaps this measurement may come into equally general use in speaking of the strength of light leather.

The four kinds of strength usually recognized by text books are resistance to tension, compression, shear, and torsion. Bursting strength is a sort of hybrid variety, which may be capable of scientific interpretation if it can be connected with one of the above varieties. This Carson[4] has been able to do. He found that the bursting strength of paper is proportional to its tensile strength in that direction of the grain in which the paper has the least elongation.

Some investigators[3] have found it worth while to apply a device to the testing machine so that the deformation can be measured simultaneously with the load.

Serious consideration has been given to the possibility of connecting bursting strength with "lastibility," the thought being that the bursting strength of upper leather should give some indication of how the leather will behave when pulled over the last in making a shoe. There appears to be some relationship—the leather must have at least a certain bursting strength or it cannot be used. However, the skill of the operator is such an important factor that it has been found difficult to establish a definite figure for lastibility.

F.S.E.C. Method for Measuring the Bursting Strength of Leather
(Proposed 1943)

Apparatus: (1) The testing instrument shall consist of a plane surface containing an aperture, 1.24 inches in diameter, which registers exactly with a similar aperture in a similar plane surface. One aperture shall be movable along the axis passing through the centers of the two apertures. (2) Means of firmly clamping the two plane surfaces together. (3) A rubber diaphragm firmly secured to the inner side of the aperture in the hydraulic chamber so as to close it off and to expand through it when hydraulic pressure is applied. (4) Means of applying hydraulic pressure through a non-compressible fluid to the rubber diaphragm. This may be done by a motor drive or a manual drive With the Mullen Tester a hand operated machine should be driven at 120 revolutions per minute to make it equivalent to the motor driven instrument (5) Means of continuously registering the pressure in the hydraulic chamber, the Bourdon type pressure gage being preferred.

Test Specimen: The selection of the test specimen shall be made in one of two ways depending on the reason for the test.

From the point of view of the tanner interested in accurate measurement of the bursting strength of a particular leather the test sample shall be cut according to the A.L.C.A. method of sampling. On the particular cuts where the size of the sample is not specified, a 6" by 8" sample shall be taken with the longer dimension parallel to the backbone. On a sample of this size twelve tests can be conveniently made, and this number shall be made. On those cuts where the method of sampling specifies the width of the sample, the sample shall be cut to the specified width and enough leather taken to perform twelve tests. The area tested shall be free from defects, and shall not have been previously subjected to clamping pressure. From the point of view of the buyer of leather, interested in the average bursting strength of a shipment of leather, test samples shall be selected as follows: Select

changing the bob of the pendulum. The force is indicated by a pointer moving over a scale graduated in per cent of the total force (corresponding to a 90° swing of the pendulum). There is also attached to the jaw-shaft another pointer moving over a scale graduated in degrees, which indicates the position of the jaws at any time. (b) the other end of the specimen rests freely against a fixed pin. This can be fixed in different positions to change the span between the jaws and the pin. (c) Provisions are made for adjusting the pointers to zero, for throwing the motor in and out of gear, and for moving the jaws by hand back to their original position. (d) The bobs supplied with the apparatus give bending moments from 0.1 to 5.0 inch-pounds in steps of 0.25 inch-pound. The pin can be fixed in a position to give spans of 0.25, 0.5, 1, and 2 inches. (e) For any specimen, the bob and the span shall be so selected that the final reading on the force scale shall be not less than 25 per cent nor more than 75 per cent.

Sample: A sample representing a shipment shall consist of seven specimens, each cut from a different piece of leather. The location from which the specimen is cut shall be as prescribed by the A.L.C.A. Each specimen shall be 0.5 inch wide, by a length equal to 1 inch more than the predetermined span. Specimens of sole leather shall be skived to the following thicknesses—men's 8 irons; women's 6 irons; finders, 10 irons. Other kinds of leather shall be tested in the thickness as received. Specimens shall be tested with the grain side down (convex when bent). All specimens shall be conditioned by the A.L.C.A. method.

Procedure: See that the apparatus is level, and properly calibrated. Place the selected bob on the pendulum and adjust the pin to the selected position (Both selections are based on preliminary experiments to meet the conditions specified above.) Clamp one end of the specimen in the jaws, being careful to see that it is in good alignment. Turn the jaws by hand until the other end of the specimen just makes contact with the pin. Adjust both pointers to zero. Throw in the motor, and let the apparatus run until the angle pointer shows that the leather has been bent through an angle of 50°. At this instant, read the force as indicated by the other pointer. Throw the motor out of gear and turn the jaws back to their original position. Replace the specimen with a new one, and repeat. Compute the average force required and convert from per cent to inch-pounds.

Report: The report shall show: (a) The average thickness of the seven specimens. (b) The span used in the test. (c) The maximum, minimum, and average (7 specimens) of the force required to bend the leather through an angle of 50°.

Note: Any specimen which does not show smooth and uniform bending during the test, thereby indicating undue lack of uniformity of structure, should be discarded and replaced by another specimen.

Cracking and Piping

When good leather is bent around a mandrel of specified diameter, there should be no visible signs of permanent cracking through the grain, if bent with the grain side out; if bent with the flesh side out, there should be no visible signs of permanent loosening of the grain in the form of wrinkles.

The size of the mandrel to use will depend upon the kind of leather being tested. A soft, thin leather can be bent through a sharper angle than a harder or thicker leather. Temperature and humidity are important, for leather will bend more easily when warm and moist than when cold and dry. Therefore all specimens for this test must be conditioned.

F.S.E.C. Method for Testing Leather for Cracking and Piping[a]
(Proposed 1943)

Cracking: This test shall be made only that part of the specimen which has been found, by careful examination, to be free from surface defects. Bend the leather, grain side out, into a loose loop and put the loop in a small vise having plain jaws not less than 2¾" wide. Place in the loop a cylindrical brass or steel rod about 7" long. Immediately above the rod insert a brass or steel bar, about 7" long, ¾" wide, and having a thickness equal to the diameter of the rod. Bend the leather around the rod and against the metal insert by slowly tightening the jaws of the vise until the rod and insert cannot be shifted

[a] Credit for this method is due R. W. Frey, U. S. Dept. of Agriculture.

by hand. Rods of different diameters are required for different kinds and thicknesses of leather. Consult the applicable specification for the correct size. See Figure 212.

Piping: The leather shall be bent through an angle of 180°, grain side in, over a mandrel of diameter shown in the appropriate specification.

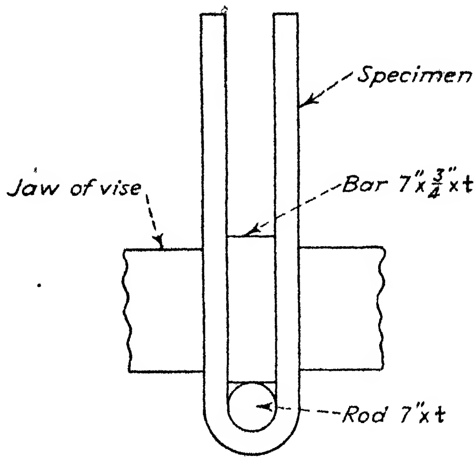


Figure 212. Equipment for testing leather for crackiness. For value of *t* see appropriate specification.

Resistance to Abrasion

Ability to resist abrasion is one of the properties which makes leather valuable. One should be careful, however, to distinguish between resistance to abrasion and resistance to wear. The latter term is the more general. It may include resistance to abrasion as well as resistance to flexing, to light, to perspiration, etc.

It has been necessary to develop two methods for measuring this property—one for heavy leather and one for light. The method for heavy leather simulates more or less the slipping and twisting of a shoe sole as one walks on an abrasive surface. The one for light leather consists of a straight rubbing action, and the abradant may be harsh or mild, depending upon the kind of information wanted; the leather may be worn through; the appearance may be impaired by destruction of the finish without seriously affecting the leather; or the method may be used as a test for resistance to crocking.

Light leather can be tested wet or dry, as desired. We have not yet been able to devise a method for measuring the abrasive resistance of heavy leather when wet, although the need for such a test is recognized.

A.L.C.A. Method for Measuring Abrasive Resistance of Heavy Leather[a]
(Provisional 1942)

Apparatus: The apparatus used shall be that shown in Figure 213, or an equivalent design. It consists essentially of a vertical wheel, 15 inches in diameter, around the cir-

[a] Credit for this method is due E. L. Wallace, Natl. Bur. Standards.

cumference of which are mounted 12 test specimens. The wheel is driven at 30 rpm. It rests on a sheet of abrasive paper or cloth which is mounted on a horizontal disc. The distance from the axis of the disc to the center line of the specimen is 5.5 inches. When the wheel is raised by means of a spring balance attached at the point A, the indicated force shall be not more than 36 lbs; when the wheel is lowered, the indicated force shall be not less than 33 lbs.

A small rotary brush and a vacuum cleaner are provided to remove loose particles from the abrasive surface.

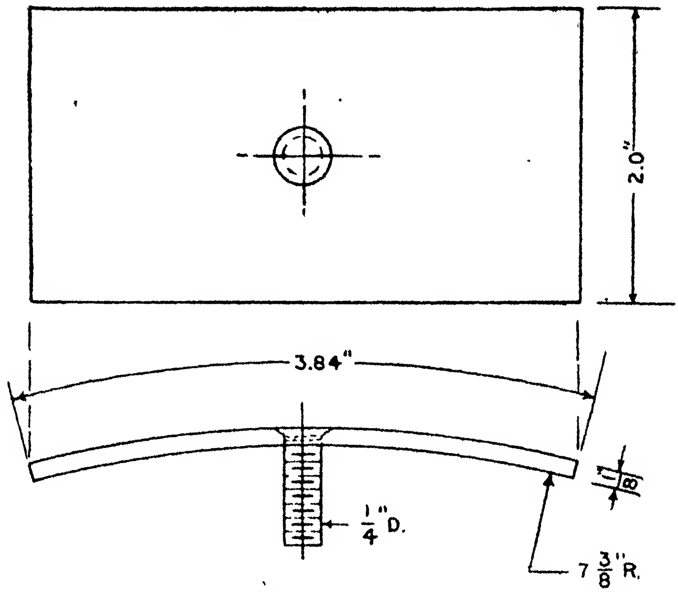
Motion of the disc is retarded by a Prony brake. This consists of a leather belt 1.25 inches wide, fastened to the frame at one end, passing around a drum 12 inches in diameter, and supporting a load of 20 pounds at the free end. The belt shall be of such quality that it will not "chatter," and that the tension at the fixed end while the machine is running shall be not more than 0.1 pound.

Figure 213. Apparatus for measuring abrasive resistance of heavy leather.

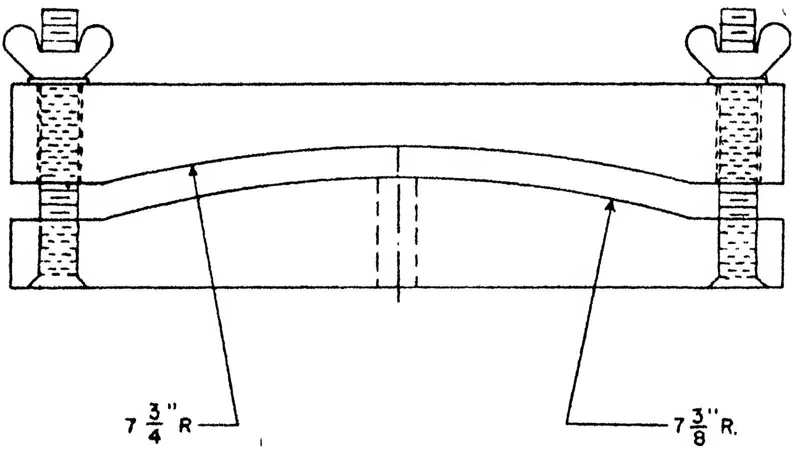
Blue prints and specifications for the machine may be obtained on request from the National Bureau of Standards.

Samples: Not less than six specimens shall be tested as representative of a sample. Where the method is to be used to measure the comparative abrasive resistance of lots of backs, bends, or sides, the six specimens shall be cut one from each of six pieces (A.L.C.A. Method of Sampling for Physical Tests).

Preparation of Specimens: Specimens shall be cut to a size 5 x 9.8 cm with a steel die. The thickness of each specimen shall be measured to the nearest 0.1 mm. All thickness measurements shall be made at five places; at the center and near the four corners of the specimen and about 0.5 inch in from the edge. Aluminum backing strips as shown in Figure 214 shall be provided. Specimens shall be attached to backing strips thus prepared



Metal backing strip



Press

Figure 214. Equipment for mounting specimens for abrasion test.

with a cellulose nitrate base cement containing only an organic solvent. Backing strips and specimens shall be bent to the curvature of the wheel in the presses shown in Figure 213 while being cemented. They shall be permitted to stand 48 hours between the time they are cemented and the beginning of the test, to ensure complete evaporation of the solvent used with the cement. Shims shall be used under the backing strips if necessary to allow for variations in the thickness of the leather.

Test Conditions: Test pieces (specimens cemented to backing strips) shall be conditioned for 48 hours and tested in an atmosphere of 65 per cent relative humidity ($\pm$ 2 per

cent) at 70° F (± 5°). If the test pieces are kept in this atmosphere during the 48 hours' drying time required above, further conditioning is unnecessary. Determine the weight of the prepared and conditioned test piece to the nearest 0.01 g and measure its thickness to the nearest 0.1 mm. After the run is completed, remove the test pieces from the wheel and again determine the weight and thickness of each. The wheel accommodates 12 pieces, that is, 2 samples of 6 specimens each. The six specimens of one sample shall be mounted next to one another, and the two end specimens shall not be included in the measurements. The abrasion index shall be computed from the loss of weight or thickness of the four specimens within the series.

Abrasive Material: The abrasive used shall be No. 50 silicon carbide, spread evenly on a cloth or paper disc 14 inches in diameter. The abrasive shall be of such size that all of it will pass a No. 30 U. S. Standard sieve, and at least 60 per cent will be retained in a No. 50 U. S. Standard sieve. The cloth backing shall be 2.85 yard, 29-inch basis drill, properly dyed and back-filled prior to coating. The paper backing shall be a good grade of rope paper having a basis weight of 130 lbs (24 x 36-480). The quantity of abrasive shall be not less than 31.1 lbs nor more than 37.7 lbs per sandpaper ream (9 x 11-480). The abrasive shall be random deposited. A new sheet of abrasive shall be used at the beginning of each test, and at the beginning of each 4,000 revolutions during the test.

End Point of Test: The test shall be continued until it is estimated by an approximate thickness measurement (which may be done without removing samples from wheel) that not less than 65 per cent nor more than 75 per cent of the original specimen is worn away. Where one or more samples wear nearly through their original thickness before the remaining specimens on the wheel have worn away approximately 70 per cent, the thin samples should be removed, weighed, replaced and the test continued until the remaining samples are worn to the required thickness.

Indices of Abrasion: The weight index is the number of revolutions required to wear away one gram of leather. The thickness index is the number of revolutions required to wear away one millimeter of leather.

A.L.C.A. Method for Measuring the Abrasive Resistance of Light Leather, Wet or Dry[a]
(Proposed 1942)

Scope: This method is designed to measure the amount of abrasion which can be withstood by light leather, wet or dry (a) without impairing its appearance or (b) without wearing through. This method is applicable to light leather only, such as upper. upholstery, bag, case, strap, etc.

Sampling: Not less than six specimens shall constitute a sample. Each specimen shall be taken from a different piece, whether skin, hide, or manufactured article. If taken from a skin or hide, it shall be taken from the location designated in the A.L.C.A. Method for Sampling Leather for Physical Testing. Each specimen shall be one inch wide by six inches long.

Conditioning: When measuring abrasive resistance dry, the specimen shall be brought to equilibrium with an atmosphere of 65 per cent ± 2 per cent relative humidity and 70° ± 5° F and tested in that atmosphere. When measuring abrasive resistance wet, the specimen shall be soaked in water for 24 hours immediately prior to testing, and a few drops of water shall be dropped on the back of each specimen every 15 minutes during the test.

Apparatus: The apparatus shall consist of a horizontal shaft carrying a number of pulleys (12 will permit the testing, of two samples of 6 specimens each simultaneously). Each pulley shall be 1½″ diameter by 1½″ face, and shall be surfaced with the abradant. The shaft shall be rotated with a reciprocal motion through 170°, making 4000 complete cycles per hour. One end of the specimen shall be fastened in a fixed clamp at one side of the pulley. The specimen shall be brought up and over the pulley and over an idler on the other side of the pulley. A dead load of 1 kg shall be attached to the free end of the specimen (see Figure 215).

Abradant: In general, for measuring abrasive resistance, dry, No. 3/0 emery cloth shall be used. For abrasive resistance, wet, No. 280A Wet or Dry abrasive paper shall be used. If the leather is likely to be worn through in less than 100 revolutions by either of the above, then the abradant used shall be No. 8 duck CCC-D-771a. If it is desired to use the machine for measuring the resistance of colored leather to crocking, then the abradant used shall be unbleached cotton sheeting, type C, Federal Specification CCC-S-281.

Procedure: Measure the thickness of each specimen at three places along the centerline cross-wise of the specimen and record the average. Mount the specimen in the

[a] Credit for this method is due R. G. Ashcraft, Endicott-Johnson Corp.

apparatus with the intended wearing surface next to the abradant. A new piece of abradant shall be used for each test and shall be replaced by a new piece after each 1000 complete cycles. Run the machine until (a) the appearance of the leather is impaired or, (b), the leather breaks, recording the number of cycles.

Report: Report the original thickness of the specimen, the number of cycles required to (a) impair its appearance or, (b), wear it through. In the latter case, the "abrasive index" shall be reported as the number of cycles required to wear through 0.001 inch of the specimen.

The report shall also state whether the test was made wet or dry, and which of the three kinds of abradant was used.

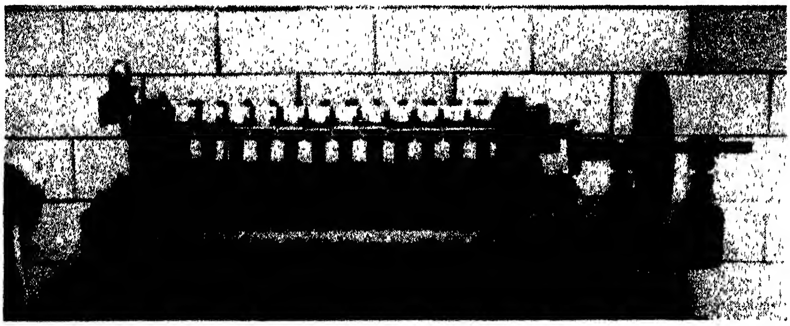


Figure 215. Machine for measuring abrasive resistance of light leathers.

Crocking

Whether or not the color will rub off is an important property of leather used for garments, hand bags, shoulder straps, etc. This property can be measured with the machine used for measuring the abrasive resistance of light leather, as just described. If such a machine is not available, the following method is recommended.

F.S.E.C. Measuring the Resistance to Crocking
(Proposed 1943)

A piece of bleached but not starched 80/80 white cotton print cloth, held firmly over the flat end of a cylindrical finger ⅝" diameter, shall be rubbed over the surface of the leather. It shall be applied with a pressure of 2 lbs and rubbed back and forth 20 times at the rate of 1 second per double stroke, each stroke being 4" long. The test shall be made with both the leather and cloth air-dry, and again when the cloth is so wetted as to contain 75 to 100 per cent water.

Bleeding

Sometimes the pigment or dye with which a leather is colored will bleed when it comes in contact with water. This defect is just as important as crocking in affecting the value of leathers for garments, handbags, shoulder straps, etc.

A.L.C.A. Method for Measuring the Resistance of Colored Leather to Bleeding[a]
(Proposed 1943)

Scope: This method is to be used to ascertain the bleeding characteristics of all kinds of colored leather whether dyed, pigmented or coated.

[a] Credit for this method is due H. L. Walker, Rohm & Haas Co.

Specimen: The specimen shall consist of two pieces of leather each approximately 4" square cut from any representative portion of the skin or hide.

Procedure: A pad of four thicknesses of 84 by 60 bleached, wide cotton sheeting (A.S.T.M. specification D-503-40-T) measuring 2" by 2" is soaked in distilled water and placed between the two leather samples having the surfaces of the leather being investigated in contact with the wet cloth. The cloth should be placed centrally on the leather so that the edge of the pad is not closer than 1" to the edge of the leather sample at any point. This preparation is then placed between two glass plates and the whole assembly placed in an oven at 158° F ± 1° F for one hour. At the end of the test the cloth is examined for discoloration.

If the cloth shows no discoloration the sample shall be reported as not bleeding.

Tackiness

Sometimes leather sticks to things when it gets warm. Upholstery leather which has been treated with an unsatisfactory finish is likely to display tackiness during a warm summer day. To test for this property, the following method may be used.

F.S.E.C. Method for Measuring the Tackiness of Leather
(Proposed 1943)

Bring the leather to a uniform temperature of 120° ± 2° F. Spread a piece of bleached cheese cloth on the coated surface and hold it under a pressure of 1 lb per sq in for 5 minutes at this temperature. Strip off the cheese cloth and note any tackiness.

Resistance to Heat

Leather, in common with other organic materials such as cotton or wool, contains a certain amount of water, which is a necessary part of its composition. Normally for leather this is about 10 per cent. This amount can be reduced somewhat without harmful effect, but if the drying is carried too far the character of the leather and its physical properties are completely and permanently changed.

Drying may be brought about by exposing the leather to a high temperature for a short time, or to room temperature for a longer time, if the surrounding air is dry enough.

In the following method, the various factors have been so selected as to produce the same effect in six days as would be produced if the leather had been stored for some years in a box on the shelf in a retail store.[8]

A.L.C.A. Method for Measuring the Resistance of Leather to Deterioration at High Temperature[a]
(Proposed 1943)

Scope: This method is used to determine the resistance of leathers to deterioration at high temperatures in the presence of moisture.

Apparatus: A diagram of the apparatus used in the test is given in Figure 216. The exposure chamber shall consist of a metal container having a capacity of 450 to 550 cubic inches, and with dimensions such that specimens for the measurement of tensile strength can be placed therein without bending. A removable cover shall be fitted to one end fastened with bolts to a flange on the container. The container shall be lined with asbestos. The temperature within the chamber shall be maintained at 100° ± 2° C. It shall be measured by means of a thermocouple placed inside a copper tube, which projects into the chamber. Inlet and outlet openings, to permit the flow of a continuous stream of air, shall be placed at opposite ends and opposite sides of the chamber. The rate of flow of the air

[a] Credit for this method is due J. R. Kanagy, Natl. Bur. of Standards.

shall be 8 to 12 ml/minute at a pressure of about 10 mm above the atmospheric pressure. It may be measured by a flowmeter attached to the outlet opening. The air shall contain enough moisture to give a relative humidity of 9 to 11 per cent in the chamber at 100° C. This may be accomplished by passing the air through water in a saturator at approximately 45° before it enters the chamber. The air shall be preheated before entering the chamber. This may be done by means of a coil in the inlet tubing as shown in the diagram. The chamber shall be provided with means for suspending test specimens in such a way as to permit good contact with the atmosphere in the chamber.

Sample: Six control and 6 test tensile-strength specimens shall be cut from each sample of leather. Each control shall be cut adjacent to a test specimen.

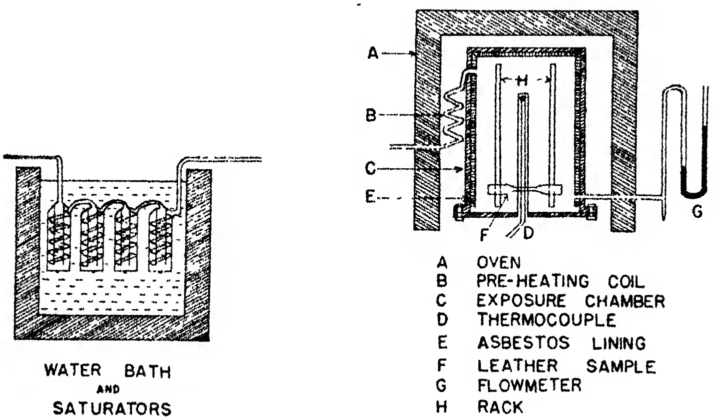


Figure 216. Apparatus for measuring resistance of leather to heat.

Procedure. Specimens for test: The specimens for test shall be conditioned at 70° F and 65 per cent relative humidity for 48 hours. They shall be placed in the exposure chamber, under the conditions of temperature and humidity described above, and allowed to remain there for 6 days. They shall then be reconditioned at 70° F and 65 per cent relative humidity for 48 hours, and their tensile strength shall be determined.

Specimens for control: The specimens for control shall be conditioned at 70° F and 65 per cent relative humidity for 47 hours and their tensile strength shall be determined.

Results: The percentage loss in tensile strength of the specimens for test shall be calculated from the difference between their average tensile strength and that of the samples for control.

Resistance to Cold Cracking

When exposed to extremely low temperature for considerable lengths of time, leather tends to become stiff and hard. If flexed while in this condition, it may crack. There is some reason to believe that leather itself is not affected by cold—that the behavior noted is related to the nature of the finished material used to coat the leather or of the grease used to stuff it.

A.L.C.A. Method of Measuring the Resistance of Leather to Cold Cracking[a]
(Proposed 1943)

Scope: This method is used to determine the resistance of coated leather to cracking at low temperatures.

Apparatus: The apparatus consists essentially of an insulated box with a compartment for Dry Ice, a fan to circulate the cold air, means for holding the sample in position, a bag of lead shot, and guide tubes through which the bag of shot is dropped on the sample. The

[a] Credit for this method is due H. C. Walker, Rohm & Haas Co.

box is also equipped with electric strip heaters and a thermoregulator in case a temperature higher than that given by dry ice is desired.

For holding the leather sample in position two brass rings are used. These are 7″ in outside diameter, 5″ in inside diameter, and ¼″ thick. The bottom ring in each pair is supported by three 1″ legs.

Sample: The sample of leather is a 7″ square.

Procedure: The sample is clamped between a pair of brass rings with that side up which will make the test simulate most closely the strain to be encountered in actual usage. For example on upholstery leather the grain side would be up, while on garment leather the flesh side would be up. The sample so held is placed on the lower shelf of the cabinet, directly below one of the guide tubes. Similarly prepared samples for comparison are placed below the other guide tubes.

The upper part of the cabinet is loaded with Dry Ice, both doors closed, and the fan started.

By means of the strip heaters and the thermoregulator the temperature is controlled at whatever point is desired. The sample is maintained at this temperature for one half hour.

The cover is removed from one of the guide tubes and a bag of lead shot weighing 2 pounds is suspended within the opening, the bottom of the bag being 12″ above the leather surface. This is then dropped freely on the sample. By means of a cord attached to the bag of shot it can be raised and used to test the next sample.

The samples of leather are removed and allowed to reach room temperature. The coated surface is examined for cracks.

If no cracks show, the test is repeated at a lower temperature. If no cracks appear at the lowest temperature attainable (−76° F), a bag of shot weighing 3 lbs or even 4 lbs may be used for further tests.

Shrinkage Temperature

If leather is heated while immersed in water, nothing will happen until a certain critical temperature is reached. Then suddenly the fibers begin to swell and to shorten. The change in dimension is indicated by an easily noticeable contraction in the length of the specimen.

The "shrinkage temperature" of vegetable tanned leather is usually below the boiling point of water, so that water alone may be used for making the test. However, when vegetable-tanned leather is immersed in water the water becomes acid, and this acidity affects the shrinkage temperature. To keep this factor constant for all leather, a small amount of hydrochloric acid is added to the water, to bring the acidity to a pH value of 3.5, which is about that of the leather itself.

The shrinkage temperature of chrome tanned leather is usually above the boiling point of water, so that water alone cannot be used for the test. A mixture of 25 per cent water and 75 per cent glycerol, having a boiling point of 117°, can be used. Since chrome leather has about the same acidity as water in equilibrium with air, no adjustment of this factor is needed.

Leather tanned by a combination of the vegetable and chrome tanning processes is regarded as chrome tanned for the purpose of this test.

A.L.C.A. Method for Measuring the Shrinkage Temperature of Leather[a]
(Provisional 1942)

Definition: When leather is gradually heated in an aqueous medium a temperature will be found at which a noticeable shrinkage occurs. This is defined as the shrinkage temperature.

[a] Credit for this method is due to E. R. Theis, Lehigh University.

Scope: This method can be used to measure the shrinkage temperature of leather of any thickness and of any kind of tannage.

Samples: Each sample shall be 0.5 inch wide by 3 inches long by the full thickness of the leather.

Liquid: For vegetable tanned leather, use water containing enough hydrochloric acid to bring the pH value to between 3.0 and 3.5. For chrome-tanned leather, use a mixture of 25 per cent water and 75 per cent glycerol. Combination tanned is to be considered as chrome tanned.

Apparatus. See Figure 35. A one liter beaker containing the appropriate liquid, two clamps for holding the sample, a stirrer, a thermometer and a heating device. The clamps are mounted vertically, 2.5 inches apart with the stationary one at the bottom. To the upper or movable clamp is attached an indicating device which will maintain the sample under slight tension, which will indicate any preliminary swelling in the sample and which will accurately detect the point at which shrinkage begins. This shall magnify the movement of the leather by at least 25 times.

Conditioning: All samples shall be conditioned to equilibrium with the standard atmosphere (65 per cent R.H., 70° F), and transferred from the conditioning chamber immediately into the beaker of liquid used in the test.

Procedure: Place the sample to be tested in the clamps, completely immerse in the liquid at room temperature, and heat the liquid with stirring, at the rate of 3 to 5° per minute. As the temperature rises, swelling of the sample occurs and the indicator may be adjusted to a reference or zero point so that eventual shrinkage can be readily detected.

In addition to the weight necessary to counter-balance the weight of the clamp or to overcome the inertia or friction of the indicating devices, it will be found advisable to add an additional weight of from 80 to 100 grams. The smaller weight will suffice for light vegetable-tanned leather such as sheepskin and the larger weight is advisable for heavy leather such as chrome-tanned side leather. In no case should a weight be used which will cause an elongation, prior to shrinkage, of more than 10 per cent.

Report: Record the temperature of the bath in degrees Centigrade at which the sample begins to shrink after the preliminary swelling.

Water Absorption

Leather when air-dry normally contains about 10 per cent of water. When immersed in water it absorbs a good deal more, the amount depending upon its affinity for water, its thickness, the temperature, the duration of immersion, the ratio of exposed surface to volume, etc. In order that results may be comparable, values for all of these factors had to be established empirically.

It is particularly to be noted that the method does not allow for the loss of material which may be dissolved out of the leather. It has been found that, under the conditions specified, the amount of such material lost is so small as to be negligible.

A.L.C.A. Method for Measuring the Absorption of Water by Leather[a]
(Proposed 1943)

Conditioning: All test pieces to be conditioned for at least 24 hours at 65 per cent relative humidity and 70° ± 5° F before testing.

Size of specimen: 50 x 98 mm rectangular.

Area exposed: The sum of the area of both surfaces and four edges.

Water: Use 10 g of water per gram of conditioned sample. Temperature of water to be 70° ± 5° F throughout the test.

Immerse the specimen grain side up in a flat-bottomed dish with not more than 150 sq cm area per specimen. (Several specimens may be immersed in a large tray at the same time.) Use small pieces of metal to prevent close contact of the specimen with the bottom of the dish.

[a] Credit for this method is due L. M. Whitmore, Leas & McVitty, Inc.

The specimen shall be fully immersed, and may be held under water by any means that does not alter the contact of the surface with water.

The specimen must be handled carefully at all times to prevent flexing or compression during the test.

Immersion periods: One-half and two hours.

Remove at the end of one-half hour. Pass a cord or wire through a small hole previously drilled near one corner of the specimen. Dry the specimen by whirling five times with a two-foot radius. Weigh. Return to the water five minutes after removal. After two hours (including the five minutes the specimen was out of water) remove, dry, and weigh as before.

Calculation: Absorption at the end of each period is computed as mgs per sq cm of exposed surface.

Absorption: $$\frac{\text{Weight (g) after Immersion—conditioned weight (g)}}{\text{exposed area in sq cm} \times 0.001}$$

Permeability to Water Vapor

Water vapor, a normal constituent of air, can go through pores which are too small to permit the passage of water. Moreover, a material having quite large pores may be able to hold water if the material itself is water repellant. For these reasons, one should not expect any relation between the two properties of a material: water permeability and water-vapor permeability.

Permeability to water vapor is a property of major importance in garment leather, upper leather, and articles of clothing in general. It is this property which permits escape of perspiration from the body, and thus contributes to the comfort of the wearer.

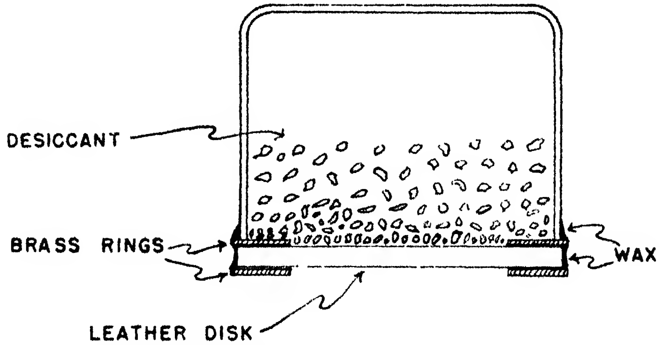


Figure 217. Equipment for measuring water vapor permeability.

A.L.C.A. Method of Measuring the Permeability of Leather to Water Vapor[a]

(Proposed 1943)

Scope of Method: This method can be used for leathers ranging from light 1¼ ounce vegetable-tanned sheepskin lining leather to 11 ounce vegetable-tanned sole leather. For leathers which are very impervious to water vapor a larger hole in the brass rings can be used.

Apparatus: The apparatus shown in Figure 217 consists of a standard tannin dish and two 20 gauge brass rings having an outside diameter of 2¾″ and an inside diameter of 1″.

[a] Credit for this method is due R. B. Hobbs, Natl. Bureau of Standards.

Sample: The sample of leather shall consist of a disk 2¾″ in diameter cut from the part of the skin under investigation. In comparing different skins the sample should be cut from the areas indicated in the Method for Sampling Leather for Physical Tests.

Assembly of apparatus: For assembling the apparatus a brass cylindrical templet over which the rings will just pass without binding, and weighing about a pound is required. A brass disk 1″ in diameter and of the same thickness as the rings is also required.

In assembling the apparatus a wax consisting of equal parts of beeswax and rosin is used.

The brass templet is centered on the sample of leather under investigation. The exposed annular surface of the sample is painted with melted wax. One of the brass rings is heated up to a temperature above the melting point of the wax and dropped over the templet onto the leather. It is held in position on the leather till the wax has solidified. The templet is then removed.

The 1″ brass disk is placed on the exposed part of the sample. The specimen is inverted, keeping this small disk in place, and the other brass ring is applied in the same way as the first. The 1″ disk is removed. The edge of the specimen is then painted with melted wax.

Hydralo (activated aluminum oxide) is used as the desiccant. The tannin dish is filled to a depth of 1″ with Hydralo. The assembly of specimen and disks is centered on top of the tannin dish and cemented to it with melted wax. This completes the assembly of the cell.

Procedure. The disk of leather under investigation is conditioned for 48 hours in the atmosphere in which it is to be tested. The sample is then removed and the cell assembled in a minimum time. The completed cell is weighed. It is then placed in the controlled atmosphere on a suitable rack with the sample down, and completely covered by the desiccant. The atmosphere is held at 95 ± 2° F and 90 per cent ± 2 per cent relative humidity.

After exposure for 24 hours the cell is weighed and replaced in the humid atmosphere. Successive weighings every 24 hours are made until the rate of absorption of water starts to fall off. This will happen when the Hydralo has absorbed about 5 per cent of its weight.

By plotting the increase in weight against the time in days a graph is obtained, the initial part of which is a straight line From the slope of this line and the area of the exposed sample of leather the transpiration rate in grams per square meter per day is calculated.

Fastness to Light

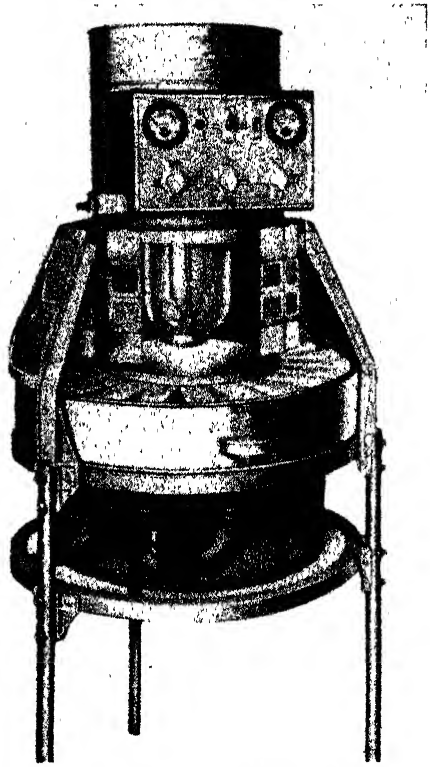
The natural color of vegetable-tanned leather is a rich yellow brown; chrome-tanned leather has a tinge of green. But for many uses, the trade demands that the leather be colored—black or white for shoes, straps, bags; red, green or blue for garment and upholstery leather. The coloring matter may be applied in the form of a dye in solution, which is absorbed by the leather, or in the form of a pigment in suspension, which is applied like a paint. In either event, the coloring matter must not rub off (see Crocking), must not run when it gets wet (see Bleeding), and must not fade when exposed to sunlight.

The following method for measuring fastness to light is written around a single testing machine. Other similar machines are on the market, which will do the work equally well, but the results obtained from the different types have not been correlated. The National Bureau of Standards is undertaking to set up a "standard light source," against which all of these machines may be calibrated. Until this work is completed, recourse may be had to the use of dyed fabrics of known fastness, which may be purchased from the American Association of Textile Chemists and Colorists.

A.L.C.A. Method of Measuring the Fastness of Colored Leather to Light
(Provisional, 1942)

Scope: This method is to be used to ascertain the fading characteristics of all kinds of colored leather, whether dyed, pigmented or coated, when exposed to light.

Apparatus: The apparatus shown in Figure 218 consists of a glass-enclosed carbon arc (such as the Fadeometer) so modified that the actinic quality of the light emitted from it approximates that of so-called standard noon sun. The arc voltage shall be between 130 and 145 volts. On DC operation the amperage of the arc shall be 13 ± ½ amperes and on AC operation 15-17 amperes.



Courtesy Atlas Electric Devices Co.

Figure 218

Racks shall be provided to support the specimens at a distance of ten inches from the arc, and so constructed that part of the sample is exposed to the light and part covered by an opaque cover. The reverse side of the sample may be covered or exposed, according to the requirements of the test. The temperature of the apparatus shall be regulated by a thermostat controlling a fan which holds the air temperature within the apparatus between 95° and 105° F. It should be noted that the temperature of the specimen will be much higher, and will depend on its color.

Humidity in the apparatus shall be provided by passing the air from the temperature regulating fan over a group of wicks, supported on wire frames, and immersed to approximately half their depth in a circular pan of water surrounding the air shaft of the temperature regulating fan. The heat from the arc assists in evaporating water from these wicks.

It is important that the wicks function properly. Distilled water only shall be used

to replenish the supply, and the wicks shall be washed and replaced as soon as they show signs of incrustation.

Specimen: The specimen shall be approximately 2½ by 4¾ inches, except when it is desired to use special holders designed to permit stepwise exposures. In such cases the sample shall be cut to fit the holder.

Procedure: Fix the specimen in the proper holder, place in position in the apparatus, and start the lamp. For most leather samples, the flesh side of the leather should be protected from the radiation, except in those cases where the leather would normally be used with both sides exposed. In cases where the flesh side is exposed, this shall be noted in the report of the test.

The sample should be examined after being in the Fadeometer for approximately six hours. If at the end of that time it shows no color difference between the exposed and unexposed portions, it should be replaced in exactly the same position in the holder, and the exposure continued. With some samples, a difference in shade may show, which will disappear after the sample has been allowed to "rest" in the dark for a short time. If, therefore, a critical evaluation is required, a rest period in the dark of two hours should be allowed before a sample is examined. The degree of fading shall be determined by visual inspection of the exposed in comparison with the unexposed area.

Comparative samples of materials designed for the same type of usage may all be exposed an arbitrary length of time, and compared for degree of fading The important point, however, is the amount of exposure required to produce an objectionable degree of fading.

The requirements for materials vary widely. For most indoor uses, an exposure period of forty hours is considered reasonable, whereas, for outdoor use, where the colored leather is to be exposed to the sun, a period of 120 hours is usually required.

For critical evaluation of colored goods, a step-wise exposure may be used, exposing the sample for a short time, covering a strip of it, continuing the exposure, covering another strip, continuing the exposure on the remainder of the sample, and so on until a number of strips having different exposures show on the sample The best procedure is to give each strip twice the exposure of the preceding one. This method differentiates colors which may show an initial fading and then very little change, from colors which show very little initial change but fail badly after the exposure has gone on for some time.

Report: The report on the fastness to light shall note the length of time the sample has been exposed, and the point at which fading first started to appear. It shall also note if both grain and flesh sides of the sample were exposed, or grain only.

The extent of fading in the sample as finally removed, shall be evaluated in terms of adjectives having meanings defined as follows:

Good: no appreciable alteration of appearance.
Fair: appreciable but not objectionable alteration of appearances.
Poor: objectionable alteration of appearance.

CONCLUSION

In this chapter, attention has been called to the desirability of measuring those physical properties of leather in which the purchaser is directly interested, and to the necessity of taking adequate samples. A formula has been presented which can be used to tell how many specimens are needed for an assumed degree of adequacy. The way of allowing for the variable moisture content of the leather is also set forth.

Methods for measuring 17 of the physical properties of leather are given in detail. Some of these have been quoted from the work of the Physical Testing Committee of the American Leather Chemists Association (A.L.C.A.), and some from the Technical Committee on Leather and Leather Products of the Federal Specifications Executive Committee (F.S.E.C.). Any particular method should perhaps be credited to the individual member of one or the other Committee who had most to do with its preparation, but all of

the methods have been changed by Committee action from the first draft presented for discussion.

The two groups are not rivals. In practically every case, the method was worked out first by the A.L.C.A., and then adopted by the F.S.E.C. However, the F.S.E.C. has usually found it worth while to make certain amendments, which later have been adopted by the A.L.C.A. In this chapter we have presented the latest editions, for which either group may have been responsible, depending chiefly on which one gave the later consideration to the method.

It must be emphasized that the methods for measuring the physical properties of leather will be in the development stage for many years to come. Experience in the use of a method will indicate its faults, which must be corrected. The development of new testing machines will provide new tools, the use of which will call for revision of the methods. Most important, a more careful analysis of the uses of leather will give a clearer understanding of the role of certain physical properties in determining the value of the leather for each use, and will probably bring to light the need for methods which do not now exist.

The buyer, the testing engineer, and the seller, should study each transaction carefully to make sure that the testing methods to be used are the most recent, and that they will give information of real value in establishing the usefulness of the leather for the intended purpose.

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